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ELECTROGRAPHIC STUDIES OF THE ISOLATED RABBIT AURICLE

BY

LEO HIRVONEN

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MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM THE INSTITUTE OF PHYSIOLOGY, UNIVERSITY OF HELSINKI
DIRECTOR: PROF. YRJÖ REENPÄÄ, M.D.

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PREFACE

It gives me pleasure to express my deep gratitude to Professor Eeva Jalavisto, M.D., who suggested the subject of this study and has generously advised me at all stages of its progress.

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L. H.

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INTRODUCTION

The physiology of the heart has been studied not only *in situ* but also in a partly or completely isolated state, and after being divided up. It is an organ with an automatic, rhythmic function and is thus an extremely gratifying object for studies in isolated state.

Numerous experiments have been made with strips of ventricular muscle. A preparation can be obtained with least injury from papillary muscle. Isolated auricles or auricular strips have also been used for observations, and these offer certain advantages as test objects over ventricular muscle. The isolated left auricle generally requires artificial stimulus for rhythmic activity. But the right auricle, which contains the uninjured sino-auricular node, contracts spontaneously.

The isolated auricle has been used to study the effect of certain physiological factors on heart muscle, and also for studies of metabolism, for pharmacological and toxicological observations, measurements of excitability, and of electrical phenomena in heart muscle. Using this preparation, the test conditions are simple and easily altered. In spite of this, loading tests and electrographic studies of heart muscle have not been carried out to any appreciable extent on isolated auricles.

The use of the isolated, spontaneously beating auricle of mammals provides a practical method for studying the mechanical and electrical responses of heart muscle, their interrelationship and the effect of the experimental conditions on the electrogram. Interest has therefore been focussed on these questions in the present investigation. Advantage has been taken at the same time of the opportunity to explain the causes of disagreement in the results of some previous investigators. As there are numerous important similarities between the electrogram recorded from an isolated part of the heart and the electrocardiogram recorded with limb leads, the changes induced experimentally in the electrogram of the isolated auricle may have both a theoretical and a practical interest.

II

REVIEW OF THE LITERATURE

1. SOME BASIC OBSERVATIONS REGARDING THE AURICLES

Martin (1904) studied the effect of potassium and variations of temperature on the isolated terrapin heart. The ventricle came to rest in a solution containing 0.12 per cent potassium chloride, although in this same solution, at the same time, the auricles continued to beat vigorously. He found that the auricles, owing to the thinness of the muscle layer, were apparently more susceptible than ventricular muscle to temperature variations.

Straub (1910) observed that the frog's auricular electrocardiogram is essentially similar to the ventricular. Bakker's (1913) results showed that electrograms of the eel's auricle and ventricle have the same form.

Langendorff and Lehmann (1906) studied the auricle of a rabbit and a cat, the preparation being perfused with a mixture of blood and Ringer's solution. No spontaneous automatic contractions were observed, though the tendency was apparent. Rhythmic activity was maintained with certain chemical, electrical and also mechanical stimuli. Erlanger (1910) studied the effect of electrical stimulation on auricular strips from various parts of the cat's heart. According to his investigations, the best response to stimulation is obtained at temperatures between 29° and 36°C. He did not consider oxygen essential to the success of his tests. Fredericq (1912) reported that a spontaneously beating isolated dog auricle produces a three-phase action current.

2. EFFECTS OF PHYSIOLOGICAL AND PHARMACOLOGICAL FACTORS ON THE MECHANICAL FUNCTION OF ISOLATED AURICLES AND PAPILLARY MUSCLE

Clark (1920) noted that the effect of a rise in temperature upon the isolated rabbit's auricle, when the frequency was allowed to vary, was a diminution in the force of contraction. The frequency increased with the temperature, but the former was not a linear nor a simple logarithmic function of the latter. He further noticed that the addition of serum after prolonged isolation of the auricle accelerated the rate and increased the force of the beat. Kruta (1949), in his turn, studied the effect of the potassium and calcium concentration of Locke's solution on the rate of the right auricle of a guinea pig at various temperatures and found that an increase in potassium retarded the rate but the rate curve was otherwise similar.

Gottdenker and Wachstein (1933) studied lactic acid formation in auricular strips of rabbit's heart under aerobic and anaerobic conditions at various pH values. Kodama (1952) also used a strip of rabbit auricle for his studies of tissue respiration under low atmospheric pressure. The slight oxygen consumption observed at low pressure increased when the pressure rose to normal, but the increase was not great enough to eliminate the oxygen debt.

Lewis and McEachern (1931) found that the isolated auricles of thyroid-treated rabbits continued to beat at a much faster rate than those of normal animals. According to Andrus and McEachern (1932), the isolated auricles of thyrotoxic rabbits are more sensitive to a lack of oxygen and to exposure to isotonic solution of sodium lactate than those of normal animals, and McEachern (1932) stated that the auricle of a thyrotoxic guinea pig consumes more oxygen than that of a normal animal. Ferranini (1936) found that injecting thyrotropic hormone into the animal a few days before the test had an effect on the isolated guinea pig auricle resembling that of thyroxin.

The effects of acetylcholine on heart muscle and its mode of action have been widely studied, using isolated mammalian auricles; e.g. Demoor (1923), Kruta (1934), Graham (1949), Burn & Vane (1949), Bülbring & Burn (1949), Webb (1950), and Burgen & Terroux (1953). Bülbring *et al.* (1952) and Burn & Kottegoda

(1953) investigated the effects of eserine and Bülbring *et al.* (1954) the cholinesterase activity in rabbit auricles and their sensitivity to eserine. Summarizing these studies roughly, it may be said that acetylcholine generally depresses the activity of the isolated auricle. In the right auricle it chiefly affects the contractility, in the left the rate. Acetylcholine does not act only as a humoral transmitter of vagal impulses; it is present in heart muscle where it plays an important part in the processes responsible for contractions. Its synthesis and the activity of the auricles are inseparably linked. Eserine, again, inhibits the effect of cholesterinase and thus also prevents the destruction of acetylcholine. According to de Elio (1947) acetylcholine shortens the refractory period of the rabbit auricle.

Trevan and Boock (1928) used the rabbit's right auricle for standardization of digitalis, working upon the assumption that digitalis causes a reversible increase in the amplitude and that this increase is proportional to the dose. The method was used later by La Barre and Gengoux (1947 and 1949) for comparative studies of cardiac glycosides. Helbichová and Kruta (1937) found that certain purine compounds increase the force of contraction and stimulate the rate of the isolated guinea pig auricle. Dawes (1946) used a preparation of isolated rabbit auricles in testing synthetic substitutes for quinidine. Demoor's and Kruta's studies of the effects of epinephrine on isolated auricles deserve attention; they will be discussed in greater detail later.

Acierno and Di Palma (1951) stated that ether, cyclopropane and chloroform depress the resting excitability of the isolated cat auricle, shorten its refractory period and weaken its contractility, thus tending to arrest spontaneous rhythmicity. On the basis of their observations an attempt was made to correlate the effects of these agents and the clinical occurrence of cardiac arrhythmias.

Ferranini (1936) made experiments to determine the effect of sodium fluoride and oxalate on an isolated auricular strip of the rabbit heart; the latter was found to be more toxic. Sachs (1937) considered that methylene blue weakens the contractions of the isolated rabbit auricle and leads to a permanent contracted state. Chang (1938) dealt with the effect of iodoacetate and fluoride on the isolated rabbit auricle chiefly from the point of view of metabolism, and Webb (1950) with the action of metabolic substrates and inhibitors.

Kruta's (1934, 1936—1938) tests on the guinea pig auricle are among the most important studies of the function of the isolated left auricle. According to him, the left auricle responded to electrical stimulation with contractions as vigorous as the spontaneous ones. Epinephrine at times caused regular contractile activity lasting for about 25 minutes when the preparation had been kept in Locke's solution for one hour, but at times it had no effect. Thyramine, histamine and barium chloride also produced contractile activity. Acetylcholine caused temporary inhibition. A left auricle exposed to the effect of histamine and barium chloride responded to 10—100 times as small doses of acetylcholine. Kruta found the preparation less sensitive to various stimuli in autumn and winter than during the other seasons. If the animals had been treated with thyroidin, every second auricular preparation contracted spontaneously — otherwise a very unusual observation in the case of the left auricle. A preparation obtained from thyroid-treated animals was considerably more sensitive to epinephrine and other stimuli.

Using an artificially stimulated auricle, Kruta studied the correlation between the rate and amplitude at various temperatures; the rate did not vary with the temperature. Each temperature reading had its optimal rate at which the amplitude or force of contraction was greatest. The colder the temperature, the lower was the optimal rate.

At constant temperature but increased rate, the duration of the contractions was prolonged to a certain maximum, to fall again later. The longest duration of contraction was obtained at a lower rate than the optimal force of contraction. The maximal length of contraction was characteristic at each temperature, and the higher the temperature the higher the rate required for maximal contraction.

The addition of calcium caused an increase in the amplitude of contraction, but a reduction in the optimal rate.

Demoor's opinion regarding the presence of a humoral factor and its importance in the mechanism of stimulation is based on his tests with isolated auricles of mammals. In his studies, alone and in association with Rylant (1922—1924, 1926, 1928, 1930, 1933, 1936, 1938, 1939 and 1941), he obtained results, some of which should be mentioned here. He found that the isolated right auricle beat spontaneously for hours if the preparation contained the

sino-auricular node. Epinephrine promoted and choline depressed its activity. If the right auricle did not include the sino-auricular node, or if the left auricle had been isolated alone, the preparation contracted at irregular intervals and the contractions were affected by neither epinephrine nor choline. If the right and the left auricle were connected by a band of tissue, they showed synchronous activity. If the connection was severed the right auricle continued to beat as before and the left performed intensive aperiodical contractions immediately or after 45 to 90 minutes. The other parts of the heart also revealed some tendency to spontaneous activity which could often be started with the aid of epinephrine. Arrhythmic series, according to Demoor, are an essential characteristic of heart muscle activity but they are subject to humoral control by the nodal tissue.

The activity of the left auricle, like that of the right, could be made regular by adding aqueous or alcoholic sino-auricular node suspension to the solution bathing the auricle. Even tissue which had not previously responded to epinephrine now became sensitized to it. This convinced Demoor that the sino-auricular node produces an agent which affects the heart muscle humorally, making it capable of contracting rhythmically. The active substance can be extracted not only from the sino-auricular node but also from other nodal tissue, for example that underlying the endocardium. If both auricles, separately isolated, were in the same solution sufficiently long, the left auricle began to beat like the right. Both reacted similarly to epinephrine and also to acetylcholine.

The active substance was not specific to any one species. If kept for 20 minutes at 53°C, its effect was more constant than when not heated. At about 60°C its rhythmicizing effect disappeared, but it retained its property of sensitizing to epinephrine. Complete loss of effect took place at 73°C.

Demoor stated that acetylcholine inhibited the rhythm produced by the active substance and caused the right auricle to perform the basic functions of heart muscle. Epinephrine stimulated the basic activity. In the presence of the active substance the activity produced by it predominated. The active substance did not destroy acetylcholine. Epinephrine and acetylcholine did not destroy one another, but they permitted the appearance of the predominant substance.

Rigler & Tiemann (1929), Brouha (1933) and Brouha & Bacq

(1936) opposed Demoor's opinions and held that rhythmic activity took place in the left auricle if enough oxygen was available. They reported that they had even removed the endocardium and fed a ventricular strip through the coronary artery, observing rhythmic activity and sensitivity to epinephrine as long as oxygen was present. The active substances, they maintained, were only break-down products of proteins with effects resembling those of epinephrine. De Roy (1936) subscribed to Demoor's theory.

One of the most interesting studies from the point of view of the present investigation is that of Little *et al.* (1953) — a test in which strips of the left atrial muscle of a dog were subjected to varying degrees of stretch. Maximal work occurred at a stretch of approximately 30 per cent. A stretch greater than this resulted in failure.

Although the papillary muscle is a part of the ventricle, it seems advisable to deal here with some studies of its function which are very closely associated with studies of the auricle.

Garb and Chenoweth (1949, 1953) noted that both a fall in temperature and increased mechanical tension widened the amplitude of the contractions. According to Garb (1951), both ammonium and calcium ions increase the contractility of papillary muscle and decrease its irritability. Therefore these two aspects of heart muscle can be made to vary independently.

Di Palma and Mascatello (1951) studied the excitability and the refractory period of the cat's heart muscle using not only auricular strips but also papillary muscle. Their results showed that on the average the auricle was less excitable and had a shorter refractory period than the papillary muscle from the same animal. Wide changes in rate and temperature did not alter the resting excitability of either auricle or papillary muscle, but both a faster rate and a higher temperature shortened the refractory period. A shift in pH towards the acid side lowered resting excitability and lengthened the refractory period. Papillary muscle maintained at a temperature of below 27°C showed summation and tetanus analogous to that seen in skeletal muscle. Auricular muscle did not show summation but under certain conditions fibrillated. The same authors (1951) studied the actions of acetylcholine, atropine, epinephrine and quinidine on the rheobasis, chronaxia, refractory period, contractility, and spontaneous activity of papillary muscle and auricular muscle.

Several pharmacological reports deserve mention. Cattell & Gold (1938) and White & Salter (1946) studied the effect of digitalis glycosides on the papillary muscle of the cat and Loubatières *et al.* (1949) on human papillary muscle. Loubatières (1949) dealt with the effects of certain heptanol and heptane derivatives on heart muscle. Green (1952) observed that a certain liver extract increased the contractility of the cat's papillary muscle in a low calcium bath. Kitto and Bohr (1953) demonstrated that cysteine, cystine, thyrosine, ascorbic acid and plasma potentiated the positively inotropic effect of epinephrine on papillary muscle.

3. ELECTROPHYSIOLOGY OF THE HEART

The Theory of the Electrocardiogram. — Koelliker and Müller (1856) demonstrated whith a frog nerve-muscle preparation that contraction of heart muscle was accompanied by electrical activity. The fact that active muscle is negative with respect to resting muscle has been known since the pioneer studies of du Bois-Reymond and Hermann. But highly conflicting opinions have been put forward regarding the character of the resulting negativity. Du Bois-Reymond (1849, 1877) considered that even in the resting state of muscle there are so-called »*electromotor molecules*» which are centres of intense chemical activity and definitely orientated. Hermann's (1874) *alteration theory* postulates that undamaged muscle is isoelectrical at rest and electrical phenomena become manifest only as a result of an internal change in the structure of the tissues due to excitation or injury.

Common to the *membrane theory* introduced by Bernstein (1902) and earlier conceptions was the assumption that active tissue is negative with respect to resting tissue. This theory embodies the idea that all excitable fibres are surrounded by a limiting membrane which is permeable to various substances in varying degrees. The behaviour of the membrane towards particles having a charge of electricity, the ions, is extremely important. Of the two most important cations of tissues, potassium ions occur almost exclusively within the fibres and sodium ions outside them. The former migrate across the membrane, but only in so far as the electrical forces binding them to the anions permit. This separation of ions gives rise to an electrical double layer with accumulation of

positively charged ions on the outer surface of the membrane and negatively charged ions on the inner surface. Such is the situation initially on the surface of each undamaged cell for which the galvanometer shows no potential differences on the application of extracellular electrodes. There is a potential difference between the inner and outer surface. When the cell is stimulated, the permeability of the membrane at the site of excitation increases suddenly. The electrical double layer disappears (depolarization), and a period follows with no charge existing across the membrane (depolarized state). After a while the pre-excitation state returns. Restitution of the positive and negative charges to their respective positions along the surface of the membrane (repolarization) occurs. Thus the excitatory process can be understood as a temporary increase in permeability of the membrane followed by a decrease.

If a curve of potential difference is recorded from an excited, arbitrarily isolated portion of undamaged heart muscle or total heart, it is disphasic. The initial deflection of the curve (*R*) corresponds to depolarization (of the ventricles in the case of the total heart) and the end deflection (*T*) to repolarization. In an ordinary electrocardiogram recorded with limb leads the *P* wave is the expression of the same event in the auricles — depolarization — as that expressed by the *QRS* complex in the case of the ventricles. The deflection produced by repolarization of the auricles, which corresponds to the *T* wave of the ventricles, is obscured by the ventricular complex, but with suitable test arrangements it can be made to appear, as for example Boden (1921) showed.

The *doublet hypothesis* was introduced by Craib (1927), who believed that — during the excitatory process — a dipole actually develops, whose negative pole is at the actual site of excitation. According to this concept, there is initially no membrane potential in the resting state, and polarization is a consequence of activity or injury. Polarization is limited to the boundary between resting and active or damaged tissue. The concept as such was supported for example by Ashman *et al.* (1940) and opposed by Gilson & Bishop (1937) and Rotschuh (1942). Macleod (1938) explained the electrogram on the basis of two dipoles: migration of one dipole gives rise to the *R* wave and the other to the *T* wave. Rotschuh accepted the »electrical doublet» only for the vector illustrating the

potential difference; the positive pole is then represented by »resting» potential and the negative pole by the maximum negativity associated with excitation. Wilson *et al.* (1933) combined the dipole concept and the membrane hypothesis. Schaefer (1951) spoke of a forced dipole, he considered the vector and dipole concepts similar and said that the doublet hypothesis is based on the membrane theory.

The purely descriptive phase in the history of the electrocardiogram was followed by a phase in which interest focussed on the explanation of individual deflections. There was a strong tendency to correlate single deflections with the activities of the various anatomic areas of the heart. For instance the *R* wave was considered the expression of activation of the base of the right ventricle, the *S* wave that of the apex of the heart, and the *T* wave that of the base of the left ventricle. Gotch (1910) believed this pathway to be determined by the embryonal development of the heart. A theory long held was that the electrocardiogram is simply the algebraic sum of the curves of both ventricles. Nicolai and Rehfisch (1908), among others, supported this opinion. Eppinger and Rothberger (1909), on the other hand, considered that the electrocardiogram is produced by the summation of the electrical events in the circular and longitudinal fibres of the cardiac muscle.

According to the *interference theory*, which originates from the studies of Burdon-Sanderson and Page (1880), a disphasic electrocardiogram is the algebraic sum of two monophasic action currents. The difference between the upstrokes of both monophasic currents produces the *R* wave. During the *S-T* segment the algebraic addition of the plateaus of the currents shows no potential difference. The difference between the downstrokes produces the *T* wave. According to this theory, all changes in the diphasic electrocardiogram can be explained by summation of the monophasic components. A monophasic current, which can usually be recorded by placing one electrode on the injured point of the heart muscle, is, in Schellong's (1933) opinion, also the current of an intact muscle element. In order to overcome certain difficulties connected with the application of the interference principle Rothschuh (1948) extended the theory to cover several pairs of monophasic currents (mehrfaches Differenzprinzip).

Einthoven (1928) recognized that the electrocardiographic

deflections occurring at any given time should not be connected with the contractions of definite anatomically limited parts of the heart muscle. The curve represents the algebraic sum of the potential differences developing in the heart at a given time. He explained the T wave on the same general basis as the *QRS* complex.

Schellong's (1937) *explanation* was that, in connection with excitation, a monophasic current is set up at the site of each particular heart muscle element. The *QRS* complex is the expression of the proximity in time of these monophasic excitatory processes and it is linked up with the function of the conducting system. As long as the excitatory state of all heart muscle elements is complete, no potential difference can be measured and the *S-T* segment is nearly isoelectrical. The *T* wave arises when the separate excitatory processes cease, and this is no uniform occurrence throughout the heart. The *QRS* complex, *S-T* segment and *T* wave are separate in principle and can be changed independently of each other.

Schaefer's (1951) *theory* on the genesis of the electrocardiogram is essentially as follows. An action potential results from summation of the potentials produced by millions of individual fibres. A single potential is represented by a vector whose direction is from negative to positive according to the direction of the propagation of the excitatory process. This is determined by the direction of the fibre which produces it. The action potential of the whole cardiac muscle is represented by an integral vector, which is the resultant of the individual vectors. The *QRS* complex is the expression of the integral vector of the cardiac action potential projected into the lead points as the excitatory process spreads. In each fibre the potential has a given duration and the potential-time-area or potential-time-integral can be measured by recording. The potential-time-integral for the heart as a whole, or the area of *QRS*, is the algebraic sum of the individual areas. This area remains constant if the number of individual fibres, their length and direction, and the rate of spread of the excitatory process remain constant. An increase in the number and length of fibres or a decrease in conductivity leads to an increase in this area. The greater the number of simultaneously activated parallel fibres, the greater the potential. If the onset of the activation of the fibres is desynchronized, the maximum potential falls but the area remains unchanged: thus the *QRS* complex widens and its amplitude is decreased.

The amplitude of the action potential of the electrocardiogram depends on cardiac factors on the one hand, and on the properties of the surrounding tissues on the other. Unspecific cardiac factors are the thickness, length, mechanical tension, number and direction of the fibres, and intracardiac short-circuiting factors, such as stasis, oedema, and necroses. Specific cardiac factors are the changes in conductivity, either in the conducting system or in the heart muscle. If the former is damaged, the myocardial elements are desynchronized. If retardation occurs in the conductivity of the myocardium, the area of the *QRS* complex enlargens. Finally there are cases which can only be explained by changes in membrane potential; this is due to the structure and metabolism of the membrane. This group includes the cases in which the area of the *QRS* complex is reduced.

The duration and vector of the *T* wave are dependent upon the manner in which the different parts of the myocardium restitute and how they are interconnected by protoplasm. The size and the vector of *T* is independent of those of *QRS*. In a single heart muscle element the part activated last restitutes last (elementarer Erregungsrückgang). This produces a negative *T* wave. The *T* wave, however, is mostly positive and this means that certain areas of the myocardium, chiefly the apical, restitute more rapidly than others (apicobasaler Erregungsrückgang).

The vector principle has gained increasing support in recent times, but the interference theory still holds its own in the German literature. Rothschuh (1952) pointed out that these two theories are not ultimately conflicting. The real difference is in the manner of presentation and the forms of expression. A unipolar electrocardiogram cannot be explained on the basis of the interference theory, but in the case of local recording Rothschuh prefers this explanation to the vector principle.

Kienle (1954) considers that the hypothesis of the vectorial characteristic of potential differences from which Einthoven (1913) developed his triangle has a completely false basis. This hypothesis, which has led to Craib's dipole concept and the vectorial interpretation of electrocardiography and vectorcardiography has no basis in physics, Kienle maintains, and is not conditioned by the electrical field of the heart but by geometric incidents. Potential difference is misrepresented as a vector entity. The electrical

field of an individual heart muscle fibre, but quite particularly that of the total heart, is a three-dimensional field of force which cannot be defined with the aid of scalar entities by tracings recorded from a few points. Thus in Kienle's opinion the concept of ventricular gradient developed by Wilson *et al.* (1934) also lacks a physical basis because of its analogy with Einthoven's vector.

In a monograph published together with Kienle, Ernsthausen (1953) introduced a new principle and concept: *the electrical picture of the heart* (elektrisches Herzbild). For this composite picture, several curves are recorded from the chest wall with adjacent electrodes having parallel axes, with lead points positioned both vertically and horizontally. The electrocardiograms thus obtained are connected up on paper, each curve in a square corresponding to the area of the chest wall from which it was recorded. The outlines of the heart are drawn in from a roentgen film. The horizontal and vertical pictures thus obtained, which these two authors consider adequate for a representation of the variations of the electrical phenomena in the heart and which they regard as expressing the behaviour of two imaginary groups of heart muscle fibres, indicate that there is a distinct boundary — or phase line — between the individual electrocardiograms with respect to the direction of their initial deflection. This line is essentially vertical in a horizontal picture and essentially horizontal in a vertical picture. The direction of the initial deflection differs on each side of it in correspondence with the counterdirected parts of the wave of excitation. The junction of the phase lines of the horizontal and vertical pictures shows the origin of the excitation wave. The method gives a characteristic picture of the electrical activity of the heart under both pathological and physiological conditions.

Electrophysiological Studies of the Isolated Heart and its Fragments. — Garb and Chenoweth (1949) recorded an electrogram in air from the cat's isolated papillary muscle. It proved of the same form as the electrocardiogram recorded with ordinary limb leads. An inversion of the *T* wave was obtained by absolute or relative anoxia. The duration of the contraction, of the *R* wave, *R-T* interval and *T* wave was doubled for each decrement of 10°C. The same authors also studied the effects of sympathomimetic amines, digitoxin, and the mercuric ion on the electrogram and mechanogram. They stated (1953) that anoxia caused inversion of the *T* wave also in

a strip of ventricular muscle of the cat. Garb (1951), studying the effect of certain cations on the electrogram and mechanogram of papillary muscle, found that the addition of potassium caused diminution of the *T* wave and widening of the *R* wave, ammonium caused inversion of the *T* wave and widening of the *R* wave, and calcium inverted the *T* wave. Both ammonium and calcium increased the contractile force. Reduction of calcium did not effect the electrogram until the calcium level was so low that no contractile force could be recorded. Then the *R-T* interval decreased and the *R-T* segment was elevated. Trautwein and Dudel (1954), using the papillary muscle of a cat, observed that the duration of the action potential and that of the isometric contraction shortened when the stimulation rate was accelerated. The amplitude of the action potential decreased slightly at a stimulation rate of over 200 per minute.

From his tests with a strip of frog ventricular muscle Schellong (1925) concluded that stretching did not influence the conduction time. As regards skeletal muscle, it is known that the conduction time is prolonged by stretching, as has been shown by Hieronymus (1913) and by Wilska and Varjoranta (1940). These observations, in Schaefer's (1951) opinion, supported his assumption that in theory there can be only slight, if any, prolongation of the *QRS* interval in cardiac dilatation. Seeman (1913) considered that stretching prolonged the duration of the upstroke of the initial deflection (*R*) of frog's heart perfused with a fluid of low conductivity. Garb and Chenoweth (1949, 1953) stated that in their studies with papillary muscle they failed to find that stretching within physiological limits produced any changes in the electrogram, although changes appeared in the mechanogram. Overstretching, on the other hand, depressed the *S-T* segment. However, Brender *et al.* (1951) noted that in a dog heart-lung preparation the *QRS* interval was prolonged when hemodynamic failure was produced by increasing the venous pressure or the arterial resistance.

Schaefer (1936) was one of those who showed that the amplitude of the action potential in skeletal muscle increases with an increase in tension. Working on the isolated heart of a frog, Straub (1910) found that the increase in mechanical tension due to increased pressure of the perfusing fluid depresses both the *R* and *T* waves. Seeman (1913) observed the same phenomenon if the heart was per-

fused with Ringer's fluid but not if the perfusate was non-electrolytic. Bogue and Mendez (1930), working with a frog heart, found that the height of the initial deflection of the electrical response diminished as the auricle or ventricle was filled or the tension of a strip of ventricle was increased. Schaefer (1951) interpreted the increase in action potential often found in hypertension as being due to mechanical tension. Rothschuh (1949) noted that a greater action potential is recorded from an empty heart than from a heart filled with Ringer's solution.

It will have been seen above that the results of tests performed are somewhat conflicting. The tests have also varied greatly in their arrangement, which makes it difficult to form a consistent overall picture. The short-circuiting effect of the surrounding medium plays an important part in both electrographic and electrocardiographic recording. Also in the living organism the heart is, as it were, in a volume conductor. Boden and Neukirch (1918) registered an electrocardiogram of the isolated mammalian and human heart without bringing the electrodes into contact with the heart. A mere change in the amount of fluid caused changes in the size, form and direction of the deflections. Craib (1927) could not explain the curves obtained in his tests in a volume conductor by the negativity hypothesis, and so he elaborated his dipole theory. According to Rothschuh (1949), the action potential of a strip of frog heart muscle, recorded in fluid, increased with the number of fibres, but this was not the case when the tracings were made in air.

III

PLAN OF STUDY

Einthoven (1928) considered study of the relationship between the mechanogram and the electrocardiogram of the heart to be of great practical importance. Schütz (1936) enumerated the following pertinent problems: (1) The inseparability of the mechanical and electrical activities, (2) the relationship between mechanogram and electrogram height, separately for diphasic and monophasic curves, (3) the time relations of the mechanical and the electrical response, and (4) the latent period of both these events.

Study of the heart, and of other hollow muscular organs, encounters a methodical difficulty (Trautwein *et al.* 1953) in that it is impossible to measure the mechanical activity of a limited area or of an individual fibre. The cardiac action potential is consequently often measured by micromethods and the mechanical activity usually by an intraventricular pressure curve. The methods of recording a mechanogram and an electrogram from a spontaneously beating auricle are comparatively much more similar.

The object the present investigation was to study the effects of mechanical stress and environmental factors on the mechanical and electrical response in heart muscle, using the spontaneously contracting isolated right auricle of a rabbit. For this purpose the experimental conditions were varied as follows:

(1) The preparation was subjected to varying degrees of load, the contractions being isotonic, and to varying amounts of stretch, the contractions being isometric;

(2) The experiments were repeated at various temperatures;

(3) The osmotic pressure, electrolytic content, and hydrogen ion concentration of the surrounding medium were modified in various ways, and tests were made also in air;

(4) Anoxia was induced in the preparation.

The height and time relationships of the mechanograms and electrograms were compared in order to find an explanation for the way in which test conditions affect the activities of the isolated auricle, in the hope that this might contribute to a clearer understanding of the corresponding phenomena in the heart as a whole.

IV

METHODS

Fifty-two adult rabbits served as test animals. Thirty-eight of these were used for preliminary tests and to develop the technique. When the technique was considered satisfactory, all the subsequent preparations were included in the series without selection. The preparation showed resistance to fatigue and its reactions were constant on repetition of the experiments sometimes at very short intervals. It was possible even to perform all of the various tests of this study on the same preparation. A material of fourteen rabbits was therefore considered sufficient for the final experiments. Each test series included observations on at least three preparations, in which case the test was repeated a few times with each preparation. The results in the definitive series were concurrent mutually and with those of the preliminary series. Additional parallel test were made with a few cat auricles. The results obtained were in keeping with those obtained with the rabbit auricles. The complete test series with cat auricles were fairly small in number, and so the results are not presented separately.

The isolated auricle was prepared as follows. The animal was stunned with a blow. The anterior chest wall was cut away, the pericardium opened, and the adipose tissue in the neighbourhood of the cranial v. cava removed. A thread was tied round this vein at about 7 mm from its junction with the right auricle. Another thread was tied at the right auriculo-ventricular groove about 3 mm lateral to the apex of the auricle. The right auricle was then removed with scissors.

The composition of Locke's solution was as follows: NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5, glucose 1.0, and H_2O to 1000.0. The pH of the solution was 8.2 and its volume in the isolation cham

ber 50 ml. A continuous stream of 100 per cent oxygen was forced into the chamber through a sintered glass plate. The temperature was regulated with the aid of a water bath. For the duration of air recording the solution was drawn off.

In the isotonic loading tests the tracing was recorded on smoked paper with an ordinary mechanical lever. The method has a certain potential error since the rate and the duration of contraction vary greatly within a temperature range of 15°C, and it is difficult to construct a recording apparatus that will function faultlessly under all conditions. For loading, a grooved wheel was connected to the axis of the recording lever and the thread passing over the wheel, was weighted. The radius of the wheel was small (2 mm) to prevent jerking of the weight. The ratio wheel radius: shorter lever arm (to which the preparation was fastened) was 1: 10, and the ratio shorter: longer arm 1: 10. Thus when a weight of e.g. 10 g was suspended from the thread, the weight acting on the preparation was 1 g. The figures in the tables indicate the latter, actual weights. The values of the amplitudes show the real changes in the length of the preparation during isotonic contractions.

The apparatus used for isometric recording consisted of a piezoelectric crystal (»Ronette» pick up set) and »Triplex» electrocardiograph (Järnhs Elektriska A-B., Stockholm, Typ EM 120) connected up through the coupling unit of a condenser (0.25 microfarad) and the resistance (10 megaohms) belonging to the pulse-registering device of the electrocardiograph. The time constant of the apparatus was 1 sec. Instead of a needle, an 18 mm long steel hook was attached to the crystal. The thread from which the preparation was suspended, was easely transferable to this hook from the lever used for isotonic recording.

The force (F) changing the shape of the crystal by means of the lever is directly proportional to the potential difference (U) produced by it in the crystal. The intensity (I) of the electric current passing through the oscillograph loop of the electrocardiograph is the quotient of the potential difference U and the resistance of the loop circuit (R). The force (F_1) acting on the thread in the oscillograph loop is proportional to the magnetic induction (B), to the current (I), and to the length of the thread (l). The moment (M) of the oscillograph is proportional to the force F_1

and equals the product of the direction moment (M_1) of suspension and of the turning angle (a) which is proportional to the deviation (a) on paper. Or, when k—k_5 are proportionality coefficients:

$$\begin{cases} F = k_1 \, U \\ I = \dfrac{U}{R} \end{cases}$$

$$\begin{cases} F_1 = k_2 \, B \, l \, I = k_3 \, I \\ M = k_4 \, F_1 = M_1 \, a \\ a = k_5 \, a \end{cases}$$

Thus:

$$a = k \, F$$

When the time factor also is taken into consideration the impulse of the force obtained is:

$$\begin{aligned} a &= kF \\ adt &= kFdt \\ \int adt &= k \int Fdt \end{aligned}$$

The area of the deviation of the mechanogram is thus the impulse of the force multiplied by k.

The proportionality coefficient between the deviation (a) and the force (F) was easiest to determine on the basis of a harmonic motion produced by a spring. The spring was stretched with a known weight (G) and then released. The recorded amplitude of the harmonic motion (a) is related to the weight G as follows:

$$\begin{aligned} G &= ka \\ k &= \frac{G}{a} \end{aligned}$$

In the equipment used, 1 gf equalled 0.9 mV. With maximal amplification 1 mV equalled 45 mm. For calculations of the impulse the required time measure is obtained direct from the recording paper.

The stretch caused by the weight of the lever used for isotonic recording (640 mg) in diastole was taken as a basis in measuring

the length of stretching. The average length of the preparation was then about 34 mm. This point appears in the tables as a stretch of 6 mm for the following reasons. Stretch was regulated with a micrometer screw of which one turn equals 3 mm or about 10 per cent of the diastolic length of the preparation. Turning the screw two full turns anti-clockwise from the basic point ensured that no stretch was exerted on the auricle in the systole either. This is the zero point of the tables. The maximum stretch used, 18 mm, is 12 mm from the basic point. In the isotonic tests a load of 2 g, corresponded to a stretch of about 8 mm and a load of 4 g to a stretch of 10—12 mm over and above the stretch caused by the lever weight. The maximum force of contraction in the series of stretching tests usually appeared with a stretch of 15 mm, and at a stretch of 18 mm some of the preparations showed diminished contractility due to overstretching; this diminution increased steeply with even a slight increase in stretching.

The observations in the loading and stretching tests were made 1 minute following an increase in stress to permit full effect but not the appearance of signs of fatique. In air recording this waiting time was shorter because the preparation quickly ceased to contract spontaneously. Using this procedure, not the slightest change in temperature was noticed during the time interval. In recording the electrogram, however, it was necessary first to wait 10—20 seconds while the fluid was drawn off the preparation and so prevent the short-circuiting influence of the fluid. In tests in which the composition of Locke's solution was varied an interval was permitted for full effect. Five minutes generally proved sufficient for this purpose if the concentration was not high enough to be injurious.

The hydrogen ion concentration was measured with the »Radiometer» pH meter 22.

The non-polarizable silver-silver chloride electrodes were prepared of silver wire insulated with shellac. One electrode was inserted into the stump of the cranial vena cava and pressed against its walls by tying a thread tightly around the stump. The other electrode was attached by thread to the other end of the preparation at a point where the wall was definitely intact. Generally a good position of the electrodes was obtained without any disturbing monophasic deformation.

The form of the electrogram recorded from a strip of isolated heart is essentially similar to the curve from the total heart recorded for example with limb leads, with the exception that the former lacks a *P* wave. The deflections of the electrogram will therefore be designated by the letters *Q*, *R*, *S* and *T*, as in electrocardiographic nomenclature. The curve obtained direct from heart muscle was termed an electrogram by Samojloff (1910), and this practice is followed here.

In measuring the size of the deflections, the *R* wave of the basic observation was taken as a unit in each test series and the other deflections and those of the other curves in the same series were indicated by a figure which shows their size compared with this unit. Thus the changes could easily be expressed as percentages.

Assessment of the *T* wave in the isotonic tests was difficult because the effect of the movements of the electrode wire attached to the freely mobile end of the preparation could not be completely eliminated, with the result that disturbing deflections often arose in the electrogram in some part of the *T* wave and after this during diastole, especially when the activity of the auricle was of great amplitude. This disadvantage was not present in isometric recording. The *T* wave was usually positive, sometimes inverted. It was often very small or even absent. Usually there was no distinct isoelectrical *S-T* segment, and the form of the curve was similar to that caused by anoxia in rabbits, as shown by Jalavisto *et al.* (1951). On the other hand, Lombard (1952) found that this curve form is characteristic of the electrocardiograms of small mammals.

Using a loupe, the *QRS* interval was easily measured to an accuracy of one thousandth of a second. The *Q-T* interval, however, could be measured only to one hundredth of a second owing to the slowly declining end of the *T* wave.

The electrogram and mechanogram of isometric contractions were simultaneously recorded with the Triplex apparatus, as shown in Figure 6.

In the statistical treatment of the material the mean error of the mean was calculated according to Fechner's formula (Bonsdorff 1943)

$$\varepsilon = \pm \frac{1.25 \ \Sigma \Delta \chi}{(n-0.2)\sqrt{n}}$$

in which $\Delta \chi$ denotes the deviation of an individual observation from the mean and n the number of observations.

The mean errors of the differences were obtained from Ritala's (1934) tables according to the formula

$$\varepsilon\ (M_1 \pm M_2) = \pm \sqrt{\varepsilon^2\ (M_1) + \varepsilon^2\ (M_2)},$$

in which M_1 and M_2 denote the averages.

V

RESULTS

A. TESTS WITH MECHANICAL STRESS

1. ISOTONIC LOADING TESTS

Mechanogram. — A detailed description of the properties and changes in the mechanogram recorded from the isolated auricle is called for here, although nothing essentially new can be expected. These changes, however, are as important as electrical events in a comparison of mechanical and electrical phenomena.

The *rate* was entirely unaffected by the amount of load, as Table 1 reveals. Slight variations are common in a biological object.

TABLE 1

RATE OF BEAT (per minute) AND LOAD

Surrounding Medium	Temperature (°C)	Load (g)						
		0	0.5	1	2	3	4	0
Locke's solution	25	61	60	61	61	62	60	60
	30	118	119	121	121	123	121	121
	35	187	188	185	186	190	191	189
	40	270	272	273	274	269	273	261
Air	30	149	151	148	150	149	147	147

The differences were not statistically significant. The situation was the same at all temperatures studied. The higher rate for the series observed in air compared with those studied in Locke's solution was due to individual variations.

The *amplitude* usually decreased as the load increased. But in some cases a small load caused an increase in amplitude. In Table 2 the amplitudes are expressed as figures.

TABLE 2

AMPLITUDE (mm) OF ISOTONIC CONTRACTIONS UNDER VARIOUS LOADS

Temperature (°C)	Load (g)						
	0	0.5	1	2	3	4	0
25	4.77	3.40	2.73	1.90	1.28	0.78	4.65
30	4.00	3.12	2.32	1.28	0.68	0.37	3.60
35	2.37	2.33	1.70	1.06	0.55	0.25	2.22
40	0.74	1.13	0.73	0.46	0.16	0.07	0.50

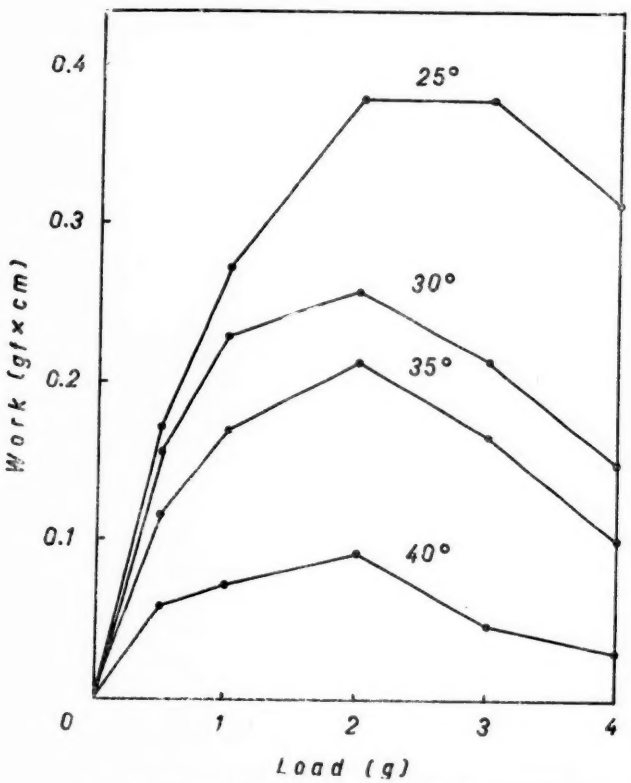

Fig. 1. — Work performed by a single contraction.

The *work performed by a single contraction* is seen in Figure 1. A feature common to the curves indicating the various temperatures is that the amount of the work increases with the load, at first steeply and later more gently. After the culmination point the curve falls more slowly than it rose.

The *work output* of the preparation was obtained from the values corresponding to the curve in Figure 1 by multiplying them with the rate at the corresponding temperature. A change in load caused no change in rate; from this it follows that the form of the output curves is similar to the curve indicating the work performed by a single contraction. Only the sequential position of the curves varies.

In air the behaviour of the amplitude and the output curves followed the same lines, provided the test series was prepared rapidly enough to avoid impairment of spontaneous activity. It was not possible to record the amplitude of an isotonic contraction without any load because the upward pressure of liquid upon the preparation — consistent with the Archimedean principle — was lacking, and consequently the effect of the weight of the preparation on the recording lever was altered.

Electrogram. — The *QRS interval*, shown in Figures 2 and 3 and Table 3 under varying conditions and with various loads, was pro-

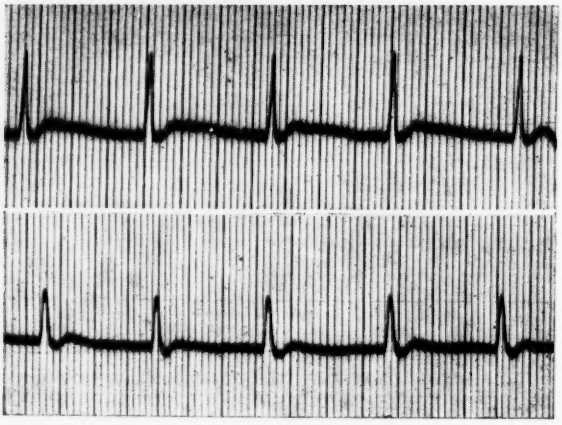

Fig. 2. — Electrograms obtained in Locke's solution at 30°C. (A) Unloaded. (B) Load 4 g.

longed as the load increased. The phenomenon was reversible although the interval did not return to its initial length immediately on termination of the test. The prolongation appeared clearly even before the maximal work output (*cf.* Fig. 1) and became more pronounced during the state of failure. The change in the *QRS*

Fig. 3. — *QRS* interval and load.

interval was similar in air and in fluid. Only the absolute figures were all higher in air. Thus, at 30°C the *QRS* interval of unloaded preparations was 0.029 ± 0.0007 sec in air and in Locke's solution 0.022 ± 0.0008 sec. The difference was 0.007 ± 0.001 sec and accordingly the *QRS* interval was about 30 per cent longer in air than in fluid.

The changes in the *R wave* in fluid differed from those in air. In the former the amplitude of the *R* wave decreased as the load increased, in the latter it rose steeply in the beginning, though later the rise eased off or there was a gradual fall (Table 4, Figs. 4 and 2).

TABLE 3

QRS INTERVAL AND LOAD

Surrounding Medium	Temperature (°C)		Load (g)					
			0	0.5	1	2	3	4
Locke's solution	25	*QRS* (sec)	0.033	0.036	0.038	0.040	0.044	0.046
		Difference from the previous value		0.0026 ±0.0006	0.0022 ±0.0008	0.0026 ±0.0007	0.0035 ±0.0011	0.0026 ±0.0011
Air	30	*QRS* (sec)	0.029	0.032	0.035	0.036	0.038	0.042
		Difference from the previous value		0.0030 ±0.0003	0.0030 ±0.0009	0.0010 ±0.0008	0.0020 ±0.0003	0.0040 ±0.0010

TABLE 4

RELATIVE AMPLITUDE OF *R* WAVE IN LOADING TESTS

Surrounding Medium	Temperature (°C)	Load (g)						
		0	0.5	1	2	3	4	0
Locke's solution	25	1.00	0.96 ± 0.03	0.91 ± 0.03	0.84 ± 0.03	0.77 ± 0.03	0.71 ± 0.04	0.82 ± 0.05
	30	1.00	0.97 ± 0.03	0.90 ± 0.04	0.89 ± 0.04	0.83 ± 0.04	0.77 ± 0.03	0.91 ± 0.04
	35	1.00	0.93 ± 0.02	0.90 ± 0.03	0.87 ± 0.04	0.87 ± 0.04	0.85 ± 0.06	0.90 ± 0.03
	40	1.00	0.91 ± 0.03	0.88 ± 0.05	0.74 ± 0.07	0.73 ± 0.06	0.69 ± 0.08	0.86 ± 0.06
	Combined (25—40)	1.00	0.95 ± 0.02	0.90 ± 0.02	0.84 ± 0.02	0.80 ± 0.02	0.75 ± 0.02	0.84 ± 0.02
Air	30	1.00	1.20 ± 0.04	1.24 ± 0.05	1.32 ± 0.07	1.28 ± 0.08	1.20 ± 0.08	0.90 ± 0.07

Multiplying the figures for the *QRS* interval and the amplitude of the *R* wave gives a product which is almost directly proportional to the *area of the QRS complex*. This method could be used instead of planimetry in view of the fact that a *Q* wave very seldom occurred in the electrograms, the *S* wave was very small and pointed, and the shape of the *R* wave was usually regular (cf. Fig. 2). As indicated in Table 5, this product remained practically constant for the

TABLE 5

RELATIVE VALUES OF THE PRODUCT OF *QRS* AND *R* IN LOADING TESTS

Surrounding Medium	Load (g)					
	0	0.5	1	2	3	4
Locke's solution	100	102 ± 2.0	104 ± 2.7	103 ± 2.8	106 ± 3.6	102 ± 4.3
Air	100	132 ± 5	147 ± 5	164 ± 7	170 ± 9	176 ± 11

QRS and *R* values recorded in fluid with varying degrees of load. The effect of the prolongation of the *QRS* interval was compensated by the lowering of the amplitude of the *R* wave. In air the situation was quite different since both factors increased with an increase in load. Even a constant *R* wave height would have sufficed to cause an increase in the product.

The *Q-T interval* remained unchanged irrespective of changes in load. The number of test series in which the duration of the *Q-T* interval could be followed throughout was small at each temperature. All these series were therefore combined. The initial value was taken as a standard with which the subsequent observations in the series were compared. The result was a difference of less than one per cent in the averages obtained with various loads.

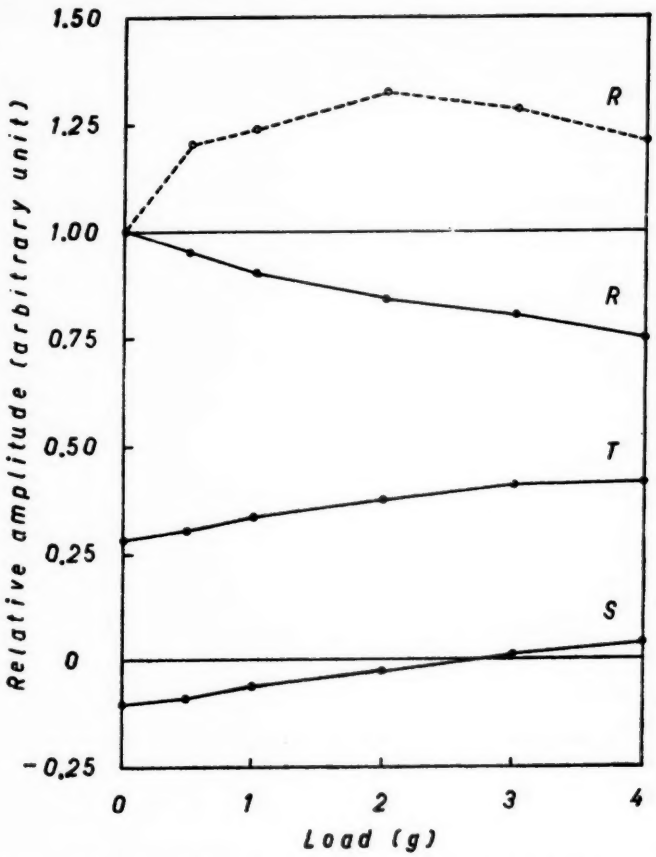

Fig. 4. — *R*, *S* and *T* in the loading tests. ——— In Locke's solution. - - - In air.

The height of the *T wave* increased with the load (Fig. 4). Although the records were made in fluid, this change was clear enough to show a statistically significant difference even between the tracings obtained with loads of 0 to 1 g and 1 to 2 g.

The *S wave* decreased in size (Fig. 4). Because of a slight monophasic deformation in some series, the average for the S wave was above the isoelectric line with the greatest loads.

2. ISOMETRIC STRETCHING TESTS

Mechanogram. — The degree of stretch did not influence the *rate* provided the resistance of the preparation was not exceeded. In the stretching tests the rate averages corresponding to the

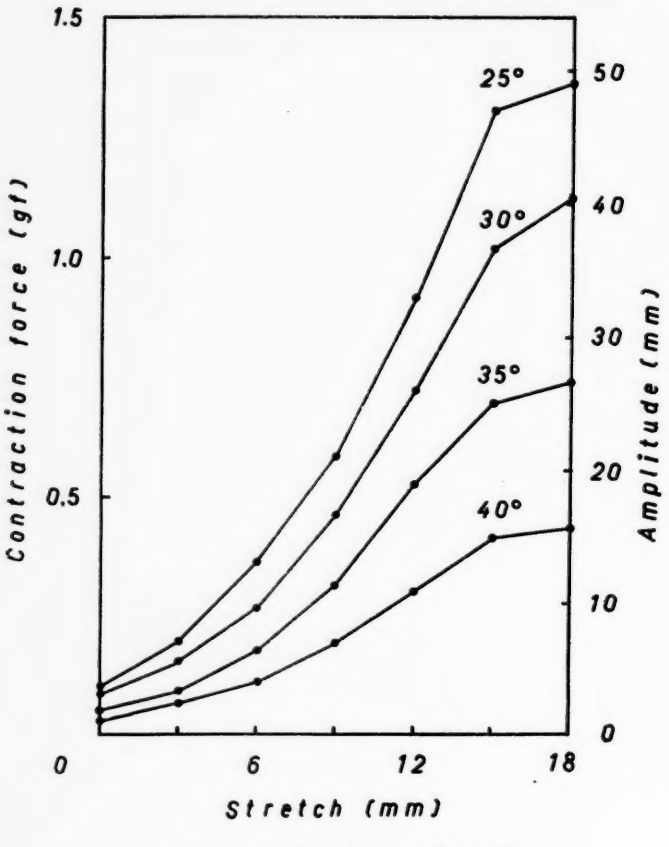

Fig. 5. — Amplitude and stretch.

temperatures 25°, 30°, 35° and 40°C were 74, 117, 169 and 242 per minute respectively.

The expression of the maximal force of contraction, *i.e.* the *amplitude* of the mechanogram, increased with the degree of stretch (Figs. 5 and 6). The tracing curves slightly at both ends. The

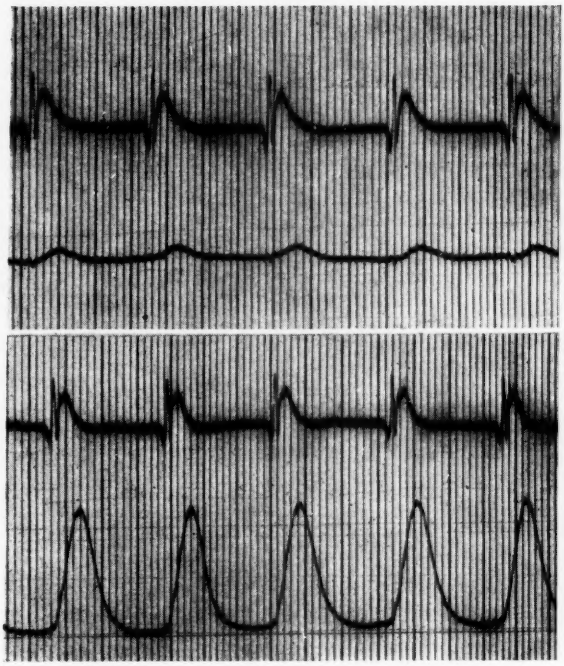

Fig. 6. — Electrogram and mechanogram at 35°C in Locke's solution. (A) Stretch 3 mm. (B) Stretch 12 mm.

initial curvature may be due to the fact that the zero point was not quite exact, and the average stretch applied at this stage is thus not directly proportional to the average stretching of the preparation. The change in direction at the end shows that the limit was reached where increased stretch caused a state of insufficiency. An average curve is considered here one where the absolute value at a stretch of 18 mm is greater than at 15 mm although in part of the test series the amplitude was smaller at the former.

In the isometric test series the work output could not be mea-

TABLE 6

DURATION OF ISOMETRIC CONTRACTION (sec)

Temperature (°C)		Stretch (mm)			
		0	6	12	18
25	Contraction	0.14 ± 0.004	0.15	0.14	0.14
	Relaxation	0.32 ± 0.011	0.32	0.35	0.39
	Total	0.46	0.47	0.49	0.53
	Difference from the previous value		0.01 ± 0.005	0.02 ± 0.007	0.04 ± 0.009
30	Contraction	0.09 ± 0.006	0.09	0.09	0.09
	Relaxation	0.19 ± 0.015	0.20	0.24	0.29
	Total	0.28	0.29	0.33	0.38
	Difference from the previous value		0.01 ± 0.005	0.04 ± 0.007	0.05 ± 0.009
35	Contraction	0.06 ± 0.003	0.06	0.06	0.06
	Relaxation	0.12 ± 0.006	0.14	0.17	0.21
	Total	0.18	0.20	0.23	0.27
	Difference from the previous value		0.02 ± 0.007	0.03 ± 0.006	0.04 ± 0.004
40	Contraction	0.04 ± 0.003	0.04	0.05	0.05
	Relaxation	0.08 ± 0.003	0.08	0.11	0.14
	Total	0.12	0.12	0.16	0.19
	Difference from the previous value		0.00 ± 0.002	0.03 ± 0.005	0.03 ± 0.006

sured because the preparation did not then perform any work. This notwithstanding, the *product of amplitude and rate* can be considered directly proportional to the output. The form of the curve indicating this product is of course the same as that of the amplitude curve, since the rate remained unchanged at varying stretches. In a given area the curve is a straight line when stretch is increased.

The *duration of a contraction* can easily be tested by isometric recording, using a crystal (Fig. 6). Two periods are distinguished: contraction and relaxation. Table 6 summarizes these observations. A study of the figures shows that the duration of the contraction period did not change at all with the degree of stretch. Relaxation, however, was slightly prolonged when stretch was increased. Thus the duration of a total single contraction is longer at a greater stretch than at a smaller.

Electrogram. — The *QRS interval* lengthened as stretch increased. This is shown in Table 7 and Figures 6 and 7. In air the

Table 7

QRS INTERVAL AND STRETCH

Surrounding Medium	Temperature (°C)		Stretch (mm)				
			6	9	12	15	18
Locke's solution	30	*QRS* (sec)	0.024	0.025	0.026	0.028	0.031
		Difference from the previous value		0.0010 ±0.0005	0.0010 ±0.0005	0.0020 ±0.0006	0.0030 ±0.0005
Air	30	*QRS* (sec)	0.030	0.033	0.036	0.039	0.043
		Difference from the previous value		0.0030 ±0.0007	0.0030 ±0.0006	0.0030 ±0.0005	0.0040 ±0.0011

same result was obtained as in fluid. Only the absolute values were higher by the former method.

The amplitude of the *R wave* decreased markedly in fluid as

TABLE 8

RELATIVE AMPLITUDE OF *R* WAVE IN STRETCHING TESTS

Surrounding Medium	Stretch (mm)				
	6	9	12	15	18
Locke's solution	0.97±0.02	0.95±0.03	0.85±0.03	0.71±0.04	0.55±0.03
Air	1.00±0.00	1.09±0.02	1.13±0.02	1.07±0.03	0.79±0.09

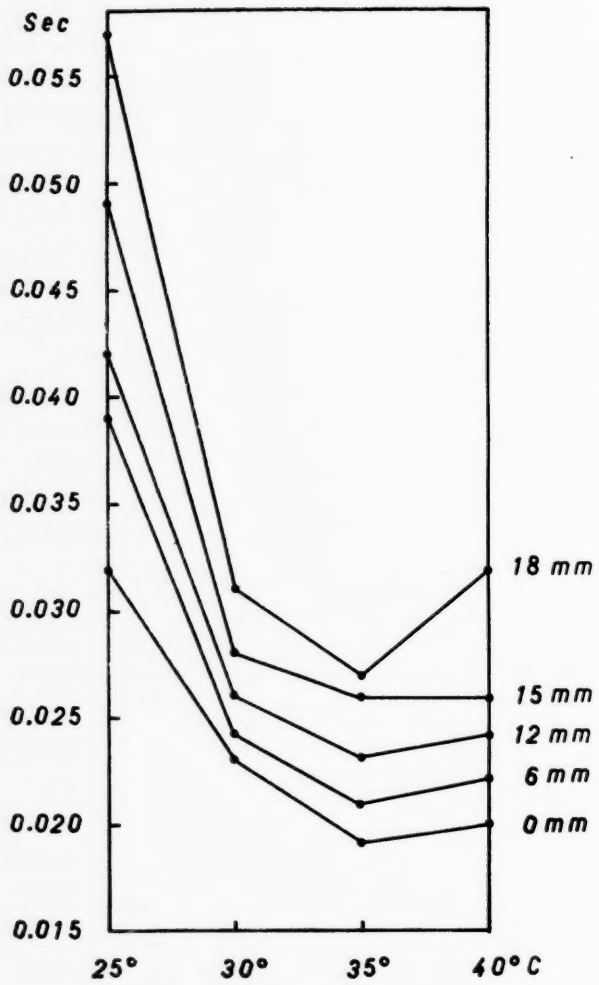

Fig. 7. — *QRS* interval and stretch.

stretch increased (Table 8). In air the change was in the opposite direction, though not very pronounced. A stretch of 18 mm was an exception.

The *product* of the figures indicating the duration of the *QRS interval* and the amplitude of the *R wave* showed a decreasing trend in fluid. In air it had a distinct tendency to increase up to a certain limit. The product increased with the amount of stretch, although not linearly (Table 9).

TABLE 9

RELATIVE VALUES OF THE PRODUCT OF *QRS* AND *R* IN STRETCHING TESTS

Surrounding Medium	Stretch (mm)				
	6	9	12	15	18
Locke's solution	100	100 ± 2	94 ± 4	87 ± 6	79 ± 6
Air	100	120 ± 3	132 ± 5	138 ± 7	112 ± 14

The existing tension did not seem to affect the *Q-T interval.* Only small variations were observed between the test series. Table 10 shows the average results at various temperatures and the relative figures in the combined series.

The *T wave* became higher with the degree of stretch when the electrograms were recorded in fluid. The *S wave,* however, decreased.

TABLE 10

Q-T INTERVAL (sec) IN STRETCHING TESTS

Temperature (°C)	Stretch (mm)						
	0	3	6	9	12	15	18
25	0.270	0.267	0.263	0.267	0.263	0.262	0.268
30	0.187	0.185	0.185	0.188	0.187	0.190	0.188
35	0.120	0.118	0.122	0.124	0.124	0.122	0.124
40	0.085	0.075	0.075	0.080	0.083	0.085	0.085
Combined	100	97 ± 1.4	98 ± 1.5	100 ± 1.1	100 ± 0.7	101 ± 1.0	101 ± 0.9

3. COMPARISON AND SUMMARY OF THE RESULTS OF THE ISOTONIC AND ISOMETRIC TESTS

The rate remained unchanged when the preparation contracted isotonically under various loads, and when it contracted isometrically on the application of varying degrees of stretch.

The change in the amplitudes differed in direction. An increase in the load led to a decrease in the amplitudes of an isotonically contracting preparation. When stretch was increased the force of contraction in an isometrically contracting auricle increased up to a definite limit. The change in both cases was almost a straight line within a certain range.

In the isotonic tests the output curve rose at first steeply and

then more slowly up to a culmen, after which the state of failure developed less abruptly than the initial rise of output. In the isometric tests the product of the maximal contraction force and the rate which can be regarded as directly proportional to the output, parallelled the amplitude curve. It thus rose with the degree of stretch to a certain maximum, to fall abruptly afterwards.

The changes in the electrogram were qualitatively similar under both test arrangements. Increased stress prolonged the *QRS* interval. The height of the *R* wave decreased in fluid and increased in air with the amount of stress. Extreme stress lowered the *R* wave in air also. The product of the figure for the *QRS* interval and the figure for the *R* wave amplitude remained unchanged or was reduced in fluid but increased with the degree of stress in air. The *T* wave increased and *S* decreased with both forms of stress. Neither loading nor stretching affected the *Q-T* interval.

B. EFFECTS OF TEMPERATURE

Mechanogram. — The observations of the effects of temperature are based chiefly on the tests with mechanical stresses (loading and stretching tests). Supplementary observations were made with unloaded preparations, recordings being made at intervals of 2.5°C.

The *rates* at various temperatures have been reported above in connection with both the loading and stretching tests. Combination of the results obtained in several test series gave the average rate: 69/min at 25°C, 118/min at 30°C, 178/min at 35°C, and 256/min at 40° C. The rate was not a linear function of temperature. Q_{10} was 2.6 between 25° and 35°C and 2.2 between 30° and 40°C, and 2.3 in the temperature series between 27.5° and 37.5°C.

The decrease in *amplitude* caused by a rise in temperature is given in Table 2 for the isotonic tests and in Fig. 5 for the isometric tests. A fairly close linear relationship obtains.

The *work output* had its optimum temperature area. The form of the output curves is similar in the isotonic tests to that indicating the work performed by a single contraction (Fig. 1) and in the isometric tests to that of the amplitude curves (Fig. 5); the tracings recorded at 30° and 35°C show fairly close agreement, and the same applies to the lower curves for 25° and 40°C. The figures in Table 11 illustrate the situation in the isotonic tests with a load of 1 g.

TABLE 11

WORK OUTPUT (gf × cm/sec) OBTAINED WITH 1 g LOAD

Temperature (°C)	Output and Difference	
25	0.28	0.22 ± 0.05
30	0.50	0.04 ± 0.10
35	0.54	0.20 ± 0.04
40	0.34	

The *duration* of an *isometric contraction* at various temperatures appears in Tables 6 and 12. The ratio of the contraction period to the cycle length was practically the same at the various temperatures; the relative length of the relaxation period was longer and the force of contraction greater at a lower temperature.

TABLE 12

DURATION OF CONTRACTION AND ITS RELATION TO CYCLE LENGTH

Temperature (°C)	Rate per Minute	Cycle Length (sec)	Duration of Contraction			
			Contraction		Relaxation	
			Sec	% of Cycle	Sec	% of Cycle
25	74	0.81	0.14	17.3	0.32	39.5
30	117	0.51	0.09	17.5	0.19	37.1
35	169	0.36	0.06	16.9	0.12	33.9
40	242	0.25	0.04	16.1	0.08	32.2

Electrogram. — The *QRS interval* had an optimum temperature at which it was shortest (Figs. 3 and 7, and Table 13). This was 35°C, even in the separate temperature series in which records were made at 32.5° and 37.5°C as well. In Table 13 the difference between the figures recorded at 30° and 35°C is 0.003 ± 0.001 sec, and that between 35° and 40°C is 0.005 ± 0.0015 sec.

TABLE 13

RELATION OF *QRS* AND *Q-T* INTERVALS TO CYCLE LENGTH

Temperature (°C)	*R-R* (sec)	*QRS*		*Q-T*	
		Sec	% of Cycle	Sec	% of Cycle
25	0.87	0.037	4.3	0.26 ± 0.006	30.0
30	0.50	0.025	5.0	0.16 ± 0.001	31.8
35	0.35	0.022	6.3	0.11 ± 0.008	31.4
40	0.26	0.027	10.4	0.08 ± 0.005	32.5

The *Q-T interval*, on the other hand, paralleled the rate: this part of the cycle remained practically unchanged. Comparison of the figures in Table 12 and 13 show the *Q-T* length to be shorter than the entire duration of a mechanical contraction. The contraction period of the mechanical systole and the *Q-T* interval are not only similarly related to the cycle but also remain unchanged on subjection to increased load and stretch.

The height of the *R wave* seemed to decrease with rising temperature, but the change was not regular. Thus the product of the figure for *QRS* interval and the figure for *R*-wave amplitude decreased as the temperature was raised.

The *T wave* proved to be highest at 35°C, *viz.* at the temperature of maximal output and most rapid conduction. No change was observed in the direction of *T* and no essential change in its form in the temperature range studied.

C. CHANGES IN SURROUNDING MEDIUM

The composition of the medium bathing the auricle was varied by degrees as regards electrolytic content, osmotic pressure and hydrogen ion concentration in both directions compared with the standard Locke's solution used in the present study.

The osmotic pressure and the electrolytic content were increased simultaneously by adding 9 per cent sodium chloride solution as such or together with potassium chloride and calcium chloride in the same proportions as these compounds were contained in the standard Locke's solution.

Addition of 50 per cent glucose solution produced an increase in osmotic pressure while leaving the electrolytic concentration nearly unchanged.

A reduction of the electrolytic concentration under a constant osmotic pressure was caused by adding a glucose solution isotonic with 0.9 per cent sodium chloride; the concentration of this glucose solution is 4.57 per cent according to Tabulae biologiae.

Both the electrolytic concentration and the osmotic pressure were lowered simultaneously with the aid of distilled water.

The pH value was increased by adding 1/10-n sodium hydroxide solution and decreased with 1/10-n hydrochloric acid.

All the test series in which the osmotic pressure increased

although the electrolytic concentration may not have changed correspondingly, are dealt with below parallelly. The test series in which the electrolytic concentration was reduced but osmotic pressure may or may not have changed are then considered separatelt. This division was made to facilitate comparison, chiefly in evaluating the amplitude of the electrographic deflections.

1. TESTS IN WHICH ELECTROLYTIC CONCENTRATION AND OSMOTIC PRESSURE INCREASED

Mechanogram. — Raising the sodium chloride concentration caused no appreciable change in *rate* until the increase exceeded 1.5 per cent. Only a slight acceleration seemed to occur. A slight change in the opposite direction appeared shortly after sodium chloride was increased to double the standard concentration (Table 14).

TABLE 14

EFFECT OF INCREASING NaCl CONCENTRATION

	NaCl Concentration (%)				Difference between Values at 0.9% and 1.8%
	0.9	1.2	1.5	1.8	
Rate (per minute)	112	118	119	104	8 ± 12
Amplitude (mm)	1.6	1.9	2.1	0.9	0.7 ± 0.2
QRS (sec)	0.023	0.022	0.020	0.018	0.005 ± 0.001
R (arbitrary unit)	1.00	0.79	0.65	0.57	0.43 ± 0.09

Addition of potassium chloride and calcium chloride together with sodium chloride caused very nearly the same results as sodium chloride alone (Table 15). Thus the presence of potassium chloride and calcium chloride did not affect the mechanogram appreciably.

TABLE 15

EFFECT OF INCREASING NaCl, KCl AND $CaCl_2$ CONCENTRATION

	NaCl Concentration (%)				Difference between Values at 0.9% and 1.8%
	0.9	1.2	1.5	1.8	
Rate (per minute)	104	112	111	102	2 ± 4
Amplitude (mm)	1.7	1.9	2.0	0.9	0.8 ± 0.2
QRS (sec)	0.024	0.024	0.024	0.024	0 ± 0.003
R (arbitrary unit)	1.00	0.79	0.50	0.42	0.58 ± 0.09

TABLE 16

EFFECT OF HYPERTONIC GLUCOSE

	Glucose Concentration (%)				Difference between Values at 0% and 6%
	0	2	4	6	
Rate (per minute)	120	121	115	100	20 ± 5
Amplitude (mm)	1.4	1.8	1.7	0.7	0.7 ± 0.2
QRS (sec)	0.027	0.027	0.029	0.031	0.004 ± 0.001
R (arbitrary unit)	1.00	1.06	0.98	0.84	0.16 ± 0.07

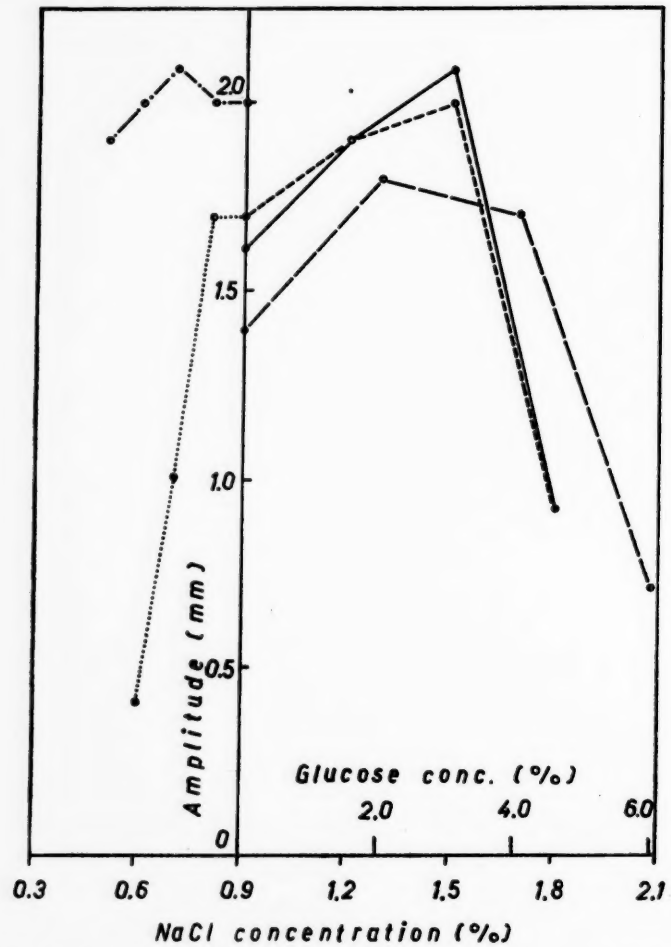

Fig. 8. — Effects on amplitude of changes in osmotic pressure and electrolytic concentration. Substances added: ——— NaCl, - - - - - NaCl, KCl and $CaCl_2$, — — — hypertonic glucose, ·—·—· isotonic glucose and ····· water.

Addition of glucose produced no definite change in rate when the glucose concentration of the solution was 4 per cent and the osmotic pressure was nearly doubled. When the glucose concentration was 6 per cent and the osmotic pressure more than double that of the original Locke's solution, there was a distinct slowing of the rate (Table 16).

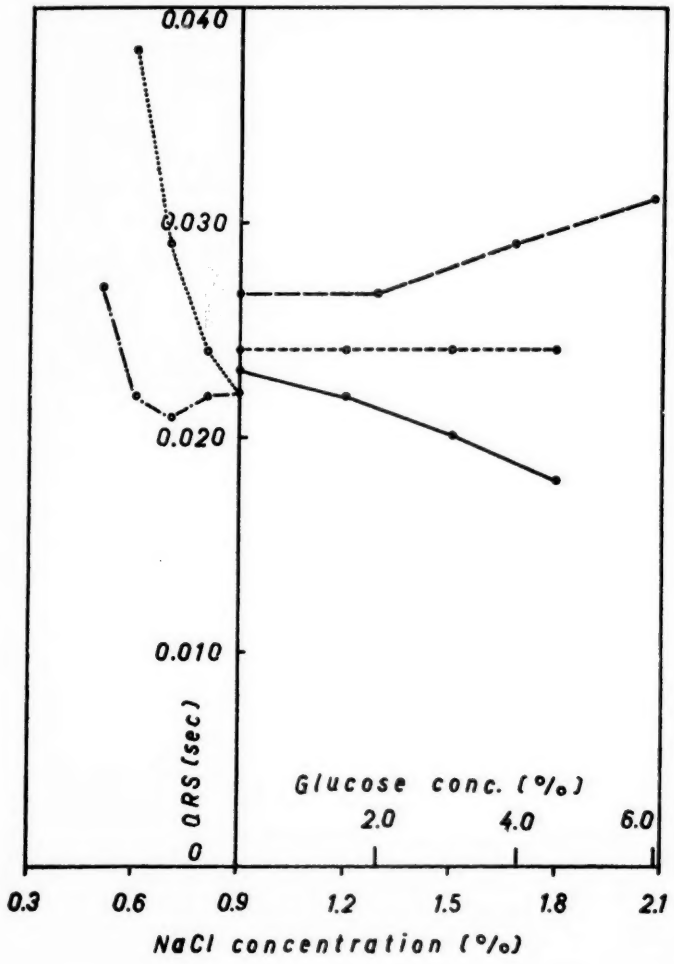

Fig. 9. — Effects on the *QRS* interval of changes in osmotic pressure and electrolytic concentration. Substances added: ——— NaCl, - - - - - - NaCl, KCl and $CaCl_2$, — — — hypertonic glucose, ·—·—· isotonic glucose and ····· water.

The *amplitude* curves were similar in form in all the cases (Fig. 8 and Tables 14—16). Initially a slight increase was observed until the sodium chloride concentration exceeded 1.5 per cent or the osmotic pressure had reached the corresponding level; then an abrupt fall followed. Increase in amplitude was regularly observed in fresh preparations especially in the glucose series; it was not seen

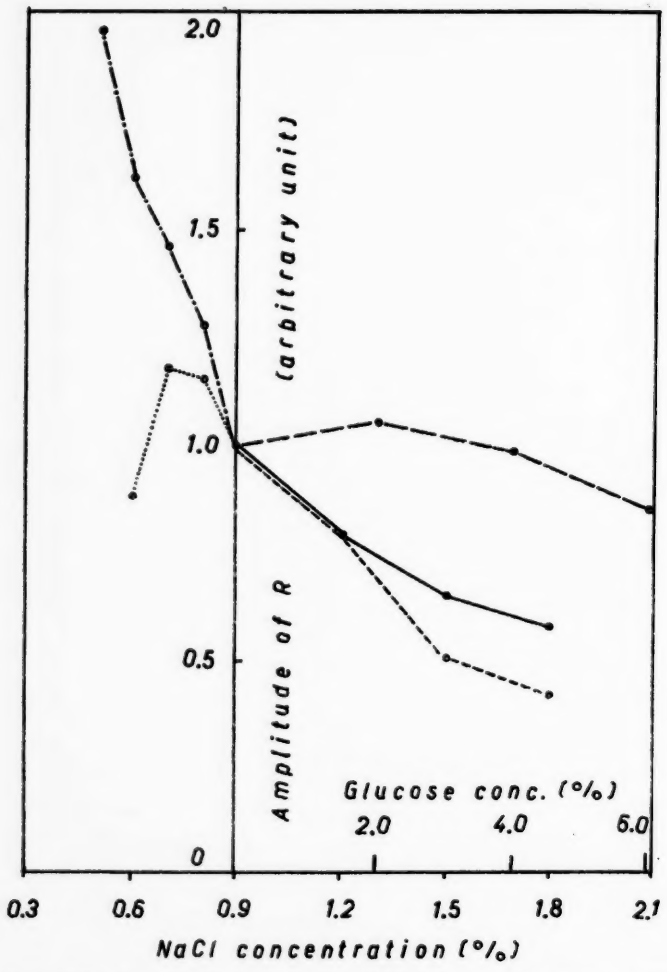

Fig. 10. — Effects on relative amplitude of the *R* weve of changes in osmotic pressure and electrolytic concentration. Substances added: ——— NaCl, - - - - - NaCl, KCl and $CaCl_2$, — — — hypertonic glucose, ·—·—·—· isotonic glucose and ····· water.

in old preparations. The amplitude was much more susceptible to an excessive salt content or osmotic pressure than the rate.

Electrogram. — The *QRS interval* remained unchanged in the test in which the sodium chloride, potassium chloride and calcium chloride concentrations were raised. When sodium chloride alone was added, the *QRS* interval shortened; at the highest glucose concentrations it was prolonged (Fig. 9 and Tables 14—16).

The amplitude of the *R wave* also showed interesting changes. It decreased as the sodium chloride concentration, either alone or together with potassium chloride and calcium chloride, was raised, in the latter case slightly more than in the former (Fig. 10). But the *R* wave remained practically unchanged if glucose was added up to the limit of 4 per cent; above this limit there was a statistically probable diminution of *R* and the mechanogram indicated greatly impaired activity.

2. TESTS IN WHICH ELECTROLYTIC CONCENTRATION AND OSMOTIC PRESSURE WERE REDUCED

Mechanogram. — When the electrolytic concentration decreased without addition of a non-electrolyte (glucose) to make up for the deficiency in osmotic pressure, the *rate* remained unchanged provided the sodium chloride concentration was 0.7 per cent (Table 17).

TABLE 17

EFFECT OF DILUTION OF LOCKE'S SOLUTION WITH WATER

	NaCl Concentration (%)				Difference between Values at 0.9 % and 0.6 %
	0.9	0.8	0.7	0.6	
Rate (per minute)	103	104	101	69	34 ± 15
Amplitude (mm)	1.7	1.7	1.0	0.4	1.3 ± 0.3
QRS (sec)	0.022	0.024	0.029	0.038	0.016 ± 0.005
R (arbitrary unit)	1.00	1.16	1.18	0.88	0.12 ± 0.10

In the series in which the osmotic pressure was maintained by means of glucose, a tendency to rate retardation appeared when the sodium chloride concentration dropped below 0.7 per cent, but there was no abrupt decrease later as when the solution was diluted with water (Table 18).

TABLE 18

EFFECT OF DILUTION OF LOCKE'S SOLUTION WITH ISOTONIC GLUCOSE

	NaCl Concentration (%)					Difference between Values at 0.9% and 0.5%
	0.9	0.8	0.7	0.6	0.5	
Rate (per minute)	132	132	125	116	110	22 ± 5
Amplitude (mm) ..	2.0	2.1	2.0	2.0	1.9	0.1 ± 0.1
QRS (sec)	0.022	0.022	0.021	0.022	0.027	0.005 ± 0.002
R (arbitrary unit) ..	1.00	1.28	1.46	1.62	1.97	0.97 ± 0.09

The *amplitude* decreased in the water dilution tests before rate retardation was observed. At a sodium chloride concentration of 0.8 per cent the amplitude was unchanged, but it then diminished rapidly (Table 17 and Fig. 8). When isotonic glucose solution was added the amplitude kept at approximately the initial level provided the sodium chloride concentration was 0.5 per cent (Fig. 8 and Table 18).

Electrogram. — The *QRS interval* was immediately prolonged in the water dilution series when the sodium chloride content dropped even slightly, and this prolongation was progressive (Fig. 9 and Table 17). With the addition of isotonic glucose, however, *QRS* remained unchanged for a long time and was not prolonged until a sodium chloride concentration 0.5 per cent was reached (Fig. 9 and Table 18).

In the water dilution series, the amplitude of the *R wave* increased until the sodium chloride content was 0.7 per cent; at 0.6 per cent there was a diminution, the amplitude of the mechanogram narrowed abruptly and the rate slowed considerably. When isotonic glucose was added the increase continued throughout the test series (Fig. 10 and Tables 17 and 18). A random observation — the electrogram was recorded in isotonic glucose solution to which only a small amount of sodium bicarbonate was added — showed an *R* wave which was about three times that recorded in the standard Locke's solution. Mechanical activity under these conditions continued only about 2 minutes.

3. CHANGES IN HYDROGEN ION CONCENTRATION

An isolated auricle contracts most vigorously in alkaline solution. The sodium bicarbonate concentration of Locke's solution

was therefore fixed at 0.5 pro mille, pH 8.2. The pH of the solution proved stable; it did not change in the dilution and concentration tests described above although no further additions of sodium bicarbonate were made.

Table 19 shows the changes in the mechanograms and electrograms caused by raising the pH and Table 20 those caused by

TABLE 19

EFFECT OF INCREASING ALKALINITY

	pH			Difference between Values at pH 8.2 and 10.0
	8.2	9.2	10.0	
Rate (per minute)	102	107	73	29 ± 10
Amplitude (mm)	2.2	2.7	1.0	1.2 ± 0.4
QRS (sec)	0.021	0.021	0.022	0.001 ± 0.002
R (arbitrary unit)	1.00	0.95	1.07	0.07 ± 0.08

TABLE 20

EFFECT OF DECREASING ALKALINITY

	pH			Difference between Values at pH 8.2 and 7.0
	8.2	7.5	7.0	
Rate (per minute)	101	94	79	22 ± 5
Amplitude (mm)	1.9	1.5	0.8	1.1 ± 0.1
QRS (sec)	0.019	0.021	0.023	0.004 ± 0.0014
R (arbitrary unit)	1.00	1.00	0.98	0.02 ± 0.01

a fall in pH. Both test series had to be started separately at the optimum pH value, since the preparation cannot be kept long at either of the extreme pH limits.

The *rate* remained almost unchanged in the pH range 7.5 to 9.2, but slowed down on either side of these limits. The *amplitude* seemed to increase initially with the alkalinity — a phenomenon observed in the initial stage also when the salt and glucose concentrations were raised. A high alkalinity (pH 10.0) resulted in a diminution of amplitude. The same applies to the fall in alkalinity below pH 8.2.

In spite of these distinct changes in the mechanogram, the

electrographic changes were slight. The amplitude of the *R wave* was unaltered within the pH limits concerned. The duration of the *QRS interval* remained the same when pH increased. But a fall in pH from 8.2 to 7.0 caused prolongation of the *QRS* interval.

It appeared that the activity of the auricle was conditioned by an alkaline pH. The optimum pH range was fairly small. Variations in either direction from this optimum caused the situation to deteriorate in both rate and amplitude. The changes in electrical phenomena under the same conditions were smaller.

D. ANOXIA TESTS

Anoxia tolerance was tested during isotonic contractions only. Different preparations differ in their resistance to lack of oxygen: spontaneous activity may cease suddenly and it is more difficult to guard against this in isometric recording. Anoxia was induced by forcing nitrogen instead of oxygen into Locke's solution. This induced much more rapid changes than the simple stopping of oxygenation. Owing to the varying anoxia tolerance it is not possible to present uniform series of anoxia tests based on the time factor. The first observation after the basic one was therefore made when nitrogen had been allowed to act for 3 minutes; recording was done slightly before the actual or probable termination of contractile activity, which occurred on an average after 8 minutes. When the stopping of oxygenation was the only thing done, the preparations occasionally contracted actively at 30° C for as much as 2 hours.

The results of the tests are presented in Table 21. It shows that

TABLE 21

EFFECT OF ANOXIA

	Initial Observation	3 min	Final Observation	Difference between Initial and Final Observation
Rate (per minute)	108	104	89	19 ± 6
Amplitude (mm)	1.8	1.3	0.1	1.7
QRS (sec)	0.024	0.025	0.031	0.007 ± 0.002
R (arbitrary unit)	1.00	0.93	0.73	0.27 ± 0.03
QRS × *R* (arbitrary unit)	100	98	101	1 ± 8

the *rate* remained nearly unchanged for 3 minutes of nitrogen flow, but was clearly slower before activity ceased. The *amplitude* decreased more abruptly: after 3 minutes there was already a clear difference, and in the last observation the amplitude was quite small. Here, too, the amplitude suffered more than the rate from the unfavourable conditions. It is clear that the changes in rate and amplitude resulted in reduced work output.

Study of the electrograms shows that the *QRS interval* was prolonged by anoxia. After 3 minutes the difference was scarcely noticeable. Before activity subsided there was a clear difference. The amplitude of the *R wave* decreased as the *QRS* interval lengthened. When the figures for *R* and *QRS* were multiplied, the *product* remained unchanged on an average. The changes in the mechanical and electrical phenomena were roughly parallel in the anoxia tests.

VI

DISCUSSION

A. COMPARISON WITH PREVIOUS REPORTS

1. *Mechanical Stress.* — The present tests showed that the *rate* of a spontaneously contracting isolated auricle remained unchanged regardless of the degree of load and stretch, if the stress was not immoderate. Knowlton and Starling (1912) made the same observation in studying the isolated total heart of mammals. In their tests the arterial resistance and venous pressure varied within wide limits. Abderhalden and Gellhorn (1920), again, noted that a sudden heavy load accelerated the rate of the ventricular strip of a frog. As the heart beats *in situ* under nervous and humoral control its rate follows other rules than that of an isolated heart strip.

A decrease in the *amplitude* of isotonic contraction with increased load may be expected as a matter of course in both heart muscle and skeletal muscle. The fact that the amplitude of the curve recorded isometrically increased with increased stretch is in accordance with Starling's (1920, 1921) law of the heart. The same phenomenon was observed by Garb and Chenoweth (1953) in papillary muscle, and for example by Santesson (1889) in skeletal muscle. Debler (1936) mentioned that in the curve illustrating an isotonic twitch the steepness of the upstroke decreased with increased initial length of the muscle, whereas in isometric contraction the upstroke increased in steepness in the beginning, and only after greater initial lengths did an abrupt fall follow.

The upstroke of the *output* curve led Abderhalden and Gellhorn (1920) to state that, when a strip of the frog's heart is loaded, the amplitude decreases less than is presupposed by the increase in load. Wertheimer (1930) found that an increase in load and output

caused an increase in the metabolism of frog heart muscle and improved its economy. Little *et al.* (1953) studied the effect of stretch on strips of dog's left atrial muscle. Their output curve agrees with that obtained in the present investigation. Maximal work occurred at approximately 30 per cent stretch, after which insufficiency set in. In the case of the preparation used in the present study, the amount of stretching caused by the load is not an equally good criterion. However, on an average the results are consistent with those reached by Little *et al.*

As for the *duration of a single isometric contraction*, the most important observation seems to be that the duration of the contraction period remained unchanged regardless of the amount of stretch. Relaxation, however, was prolonged with increased degrees of stretch.

Kruta (1937) studied the left auricle of the guinea pig heart using artificial stimulation; he thus had an opportunity to observe the amplitude and the duration of contraction under conditions in which the rate was independent of the temperature. He measured only the duration of the contraction period. It appeared that, when the rate was accelerated, the duration — with one exception occurring at the very beginning — was always prolonged to a certain limit and then diminished. The maximum duration of a contraction was obtained at a slower rate than the maximal force of contraction.

Lombard and Cope (1926) measured the length of systole of the left ventricle of man by means of a carotic sphygmogram and found that it was not influenced by changes in systolic, diastolic or pulse pressure. According to Hafkesbring and Ashman (1928), mechanical systole was prolonged by procedures which increased the diastolic volume of the ventricles. The results of de Burgh Daly (1923) showed that the duration of the mechanogram recorded from the isolated ventricle of the turtle heart lengthened as the pressure of the perfusing fluid rose. — Santesson (1892) and Debler (1936) found that the contraction period of skeletal muscle increased to a certain extent with increasing load.

In the present study the *QRS interval* of the rabbit auricle lengthened with increased mechanical stress. This observation parallels that of Brender *et al.* (1951), who showed that in a heart-lung preparation *QRS* is prolonged during hemodynamic failure. It

differs, however, from the result obtained by Schellong (1925) in studying frog's heart muscle.

Many authors have studied the effect of stretch on conduction in skeletal muscle. Hoffmann (1913) concluded from his experiments on frog's sartorius muscle that the propagation velocity of the negativity is independent of the length and tension of the muscle in tetanus. Hieronymus (1913) used sartorius muscle treated with curare and showed that the time taken for the impulse to travel from one end of a muscle to the other increased proportionately with the stretch. Wilska and Varjoranta (1940) studied the transverse abdominal muscle of the frog, demonstrating that the time required for the action potential to advance from one end of a muscle fibre to the other was prolonged, but the prolongation was proportionately less than the amount of stretch, indicating an increase in velocity. According to Martin's (1954) studies, the conduction velocity in the frog's sartorius muscle remained nearly or completely unchanged within certain limits. Håkansson (1954) found that in isolated frog muscle fibres the propagation velocity of the action potential remained unchanged up to a stretch of 60 per cent above equilibrium length, after which the velocity increased. Thus all the above authors consider that the time taken by the excitatory process to travel from one end of a skeletal muscle fibre to the other increases as a result of stretch; this applies also to the isolated rabbit auricle.

The *Q-T interval*, as well as the contraction period of the mechanogram, was unaffected by stress in the present study, although the total duration of a mechanical response was prolonged with increased stretch.

Schaefer states in his monograph dealing with electrocardiography that mechanical and electrical systole may vary in length, and the latter may even be the longer of the two. Seeman (1913), on the basis of his experiments on the isolated frog heart, said that the duration of the electrogram did not exceed that of a mechanogram. Hafkesbring and Ashman (1928) found that electrical systole did not lengthen as the diastolic volume of the ventricles was increased, although mechanical systole became longer.

According to the present study, the *Q-T* interval of the rabbit auricle is longer than the contraction period but shorter than the total duration of a single contraction. Blair *et al.* (1941) reported that in

turtle heart strips the duration of isometric contraction, as measured from half contraction to half relaxation, was related over a wide range to the *Q-T* interval by which they meant the time between depolarization and repolarization. The *Q-T* interval, according to them, coincides with the absolute refractory period. They think that repolarization arrests the contractile process in the muscle, from which they conclude that the electrical processes control the mechanical rather than the mechanical, the electrical. Schütz (1934) stated that the end point of a monophasic action potential occurred during relaxation. The duration of a monophasic action potential, again, coincided exactly with the *Q-T* interval of a diphasic curve (1932). In the opinion of Schellong and Schütz (1928) the action potential recorded from the ventricle and in Buchthal's (1931) opinion that recorded from the auricle terminate at exactly the same time as the absolute refractory period. The studies of Di Palma and Mascatello (1951) demonstrated that in a cat's isolated papillary muscle mechanical systole and the refractory period were about equal in length, but in the auricle mechanical systole was markedly shorter.

In the present writer's tests with mechanical stresses the changes in the *amplitude of action potential* differed in fluid and in air. In the former stretch caused a decrease and in the latter an increase up to a certain limit.

Even Lamansky (1870) and Bernstein (1897) in their time noted that the amplitude of the action potential of skeletal muscle increased with stretch. Schaefer (1936) made the same finding with a more modern technique. In Håkansson's (1954) experiments the amplitude of the action potential in an isolated muscle fibre remained almost unchanged as stretch reduced the mechanical response.

Krayer and Schütz (1932) induced changes in the work output of a dog heart-lung preparation, varying the arterial resistance and venous pressure and using barbiturate acid derivatives to depress activity and epinephrine to increase it. They found no correlation between mechanical response and the recorded monophasic action current, and the height of the latter remained unchanged. According to Arbeiter (1921) there were parallel changes in the size of the deflections of the mechanogram and electrogram of a perfused turtle heart. Eismayer and Quincke (1930) made experiments on a frog's heart and noted that the height of the initial deflection of the

action current decreased with the growth of the diastolic volume. Straub's (1910) observation was similar. Seeman (1913) observed that increased pressure of the perfusing fluid caused a diminution only if it contained electrolytes. Otto (1928) compressed the v. cavae of a dog after isolating the heart from nervous control; the electrocardiographic deflections decreased. Schaefer (1944) reports that, by compressing the cat's ascending aorta, which doubled the blood pressure, he obtained an increase in the *QRS* amplitude in one lead; this he considered to be due chiefly to mechanical tension.

Both the results in the present tests and the examples from the literature show that, from the point of view of changes in the amplitude of action potential, the conductivity of the surrounding medium or of the contents of the heart is decisive and may essentially alter the changes caused by mechanical stretching alone.

2. *Temperature.* — Since the time of the first observations regarding the effect of temperature on the heart (e.g. Budge 1846) it has been known that a rise in temperature accelerates the heart *rate.* Numerous studies have been carried out to determine the correlation between rate and temperature. Knowlton and Starling (1912) and Badeer (1951) showed that the rate of the isolated mammalian heart varied directly with the temperature, the correlation curve being a straight line. Kanitz (1913) and Snyder (1913) considered that the curve representing the influence of temperature upon the heart rate of mammals is exponential in character. Gellhorn (1924) reported the same for a frog heart strip. Barcroft and Izquierdo (1931) studied the relation of temperature to the pulse rate of the frog. Their results varied seasonally. Kruta (1937) demonstrated that the artificially stimulated left auricle of a guinea pig had an optimum rate at which the force of contraction was maximal. The optimum rates at various temperatures followed van't Hoff's formula in such a way that Q_{10} averaged 2.8.

According to Clark (1920), the rate of an isolated mammalian auricular preparation is neither a linear nor a simple logarithmic function of temperature, and moreover Q_{10} is not even constant. The values obtained by him were 3.1 at 20°—30°C and 2.2 at 30°—40°C. In agreement with these values are the results obtained in the present study: Q_{10} was 2.6 at 25°—35°C, 2.3 at 27.5°—37.5°C and 2.2 at 30°—40°C.

The *amplitude* of contraction, or the contractile force, decreased with increased temperature in the present tests. Doi (1920) observed this phenomenon in cardiac and skeletal muscle. Clark (1920) reported that this occurred in the isolated auricle of mammals if the rate was allowed to change freely with the temperature. If, however, the rate was maintained at the same level by artificial stimulation, the contractile force increased as the temperature rose. In Kruta's (1937) tests the maximal force of contraction decreased with a rise in temperature.

According to Eckstein (1920), the amplitude of a single isotonic contraction decreased in both heart and skeletal muscle as the temperature rose, but in smooth muscle the amplitude grew. Sugai (1930) observed the same mode of reaction in frog skeletal muscle but an opposite one in the pale skeletal muscle of a guinea pig. Bernstein (1908) stated that in both isometric and isotonic contraction the amplitude and the mechanical efficiency increased as the temperature fell.

In the present experiments the *work output* of the isolated auricle was greatest at 30° and 35°C. A fall in temperature was not accompanied by a sufficient increase in contractile force to compensate the weakening from rate retardation, and the rate acceleration with a rising temperature did not make up for the effect of the weakening of the contractile force. Although the test series were not sufficiently complete to allow determination of the accurate optimal temperature for maximal work output, they did show that the isolated auricle has a maximal output at a temperature below but close to the rabbit's body temperature.

There is general agreement that the isolated heart and heart strips have an optimal activity at a temperature below that of the body. This applies to the heart isolated by Langendorff's (1897) method. Taussig and Meserve (1925) stated that the optimum temperature for contractile activity of a strip of mammalian ventricle is 32° to 35°C. In fact, a temperature closer to 30°C than to the body temperature has often been used in studies of the isolated right auricle (e.g. Burn and Vane 1949). Kruta (1938) used the product of amplitude and rate to indicate work output; the absolute figure for optimal work output was near body temperature. His test arrangements gave the maximal output at a more rapid rate than that corresponding to the optimum amplitude at each temperature.

Knowlton and Starling (1912) stated the output of the isolated heart to be independent of temperature within wide limits.

Several authors have studied the *duration of contraction* at various temperatures; Hirvonen and Jalavisto (1954), who used the isolated right auricle of a rat, Garb and Chenoweth (1949) with cat papillary muscle, and Kruta (1937 and 1938) with the guinea pig's left auricle. These studies consistently showed a shortening of duration with rising temperature. Kruta's view was that, at each temperature, an artificially stimulated preparation has a characteristic rate which corresponds to the maximal duration of contraction. This rate changes neither linearly nor logarithmically with rising temperature, but tends to conform to Belehrádek's (1935) empiric formula.

In the present investigation the duration of the contraction period of isometric contraction was prolonged with a fall in temperature in such a way that its relation to cycle length remained unchanged. Relaxation, however, occupied a greater proportion of the cycle, and at the same time the force of contraction increased. The difference from the isolated auricle of the hedgehog is interesting: in the latter a rise in temperature prolonged both the contraction and relaxation periods in relation to cycle length in the temperature range 10°—35°C (Hirvonen 1955).

According to Ruskin *et al.* (1949), changes in temperature affect the intraventricular *conduction time* much less markedly than that between the atria and ventricles. Wendhut (1951) heated and cooled the sinus of a frog heart; he obtained a change in rate but did not notice any correlation between the duration of the *QRS* interval and the rate. Giller *et al.* (1952) induced hyperthermia in a dog and found no change in the *QRS* interval up to 42°C. Meda (1952) reduced the body temperature of a guinea pig to 22°C and recorded a monophasic action current. It appeared that both depolarization and repolarization were retarded. Sarajas (1954) reported that when the body temperature of a hedgehog fell during hibernation from about 37°C to about 7°C, the *QRS* interval was prolonged by 425 per cent, the corresponding average prolongation of cycle being 775 per cent.

The present study of the isolated rabbit auricle showed distinctly that the absolute figure for the *QRS* interval has its optimum temperature. This is below the rabbit's body temperature, and the

maximal output is also obtained below body temperature. In skeletal muscle the situation is different. Wilska and Varjoranta (1940) showed that the conduction velocity in muscle fibres increased continually although not linearly with increased temperature; at 36°C it was more than five-fold the velocity at 0°C.

The *Q-T interval* shortens with heating, lengthens with cooling. This observation was made by Ruskin *at al.* (1949) from a study of isolated rabbit hearts. According to them the *Q-T* interval lengthened parallelly with the degree of cooling: moderate or marked elevation of temperature prolonged it at various rates of artificial stimulation. Giller *et al.* (1952) found that a rise of temperature shortened the *Q-T* interval in the electrocardiogram of a hyperthermic dog more than can be accounted for by the rise of rate. Nahum *et al.* (1941) stated that a change of the temperature due to local manipulation of the heart surface caused a change in the duration of the monophasic action current.

The present investigation shows that the *Q-T* interval remained unchanged in relation to cycle length, although the rate of the isolated auricle varied with the temperature. There is a difference here from the state observed in man when the heart rate increases. Many authors, such as Fridericia (1920) and Hegglin & Holzmann (1937), have expressed the normal length of *Q-T* by means of a formula. According to all the formulas, the *Q-T* interval increases in relation to cycle length as the rate is accelerated. According to Hegglin and Holzmann's formula, *Q-T* occupies 32 per cent of the cycle at a rate of 40/min and as much as 63.5 per cent at a rate of 150/min. It is clear that the conditions in a human heart beating at various rates cannot be considered similar to those in the isolated rabbit auricle, in which the change in rate is caused only by changing the temperature within wide limits. This notwithstanding, a comparison is of interest. Sarajas (1954) has shown that in the hedgehog the ratio *Q-T* : *R-R* decreased during hibernation.

The vegetative nervous system is capable of influencing the proportion of cycle occupied by electrical systole. Hofmann (1926) observed that stimulation of the cardiac vagus shortened the duration of the monophasic action current although the rate was maintained at the same level. In Savilahti's (1941) exercise tests the rate and the *Q-T* interval were not directly related. According to Linko's (1951) study, however, the *Q-T* interval of patients with

neurocirculatory asthenia did not differ appreciably from that of normal subjects.

Many authors have shown that the *T wave* is sensitive to temperature changes. Seeman (1913) obtained inversion of *T* in the frog heart with cold. On heating, the *T* wave rose at first and later declined when the rate increased. Hoff and Nahum (1941) heated and cooled the two ventricles of a dog separately, and so produced changes in *T*-wave direction in certain cases; this observation they used in support of the view that *T* consists of the difference between the terminal portions of a dextro- and levogram. Giller *et al.* (1952) having induced hyperthermia in dogs, observed sometimes increased, sometimes decreased, and sometimes inverted *T* waves. The results of Ashman *et al.* (1945) showed that in the normal heart at ordinary heart rates the *T* wave is mainly due to differences in the time required for the repolarization of different muscle elements. The changes of cycle length reduced these differences and thus caused changes in the form and amplitude of the *T* wave.

In the present study the *T* wave was highest at 35°C. The small number of the series limits the certainty of the conclusion. It is interesting, however, to note that the highest *T* wave occurred in the temperature range corresponding to the maximal work output. Inversion of the *T* wave did not occur in the temperature range 25°—40°C.

3. *Changes in the Composition of the Surrounding Medium.* — Since the time of Ringer's (1882) classic publication, numerous studies have been made regarding the effects of *electrolytes*, especially potassium and calcium, on heart muscle. Some of these may be reported as examples: Martin's (1904) study of terrapin heart, Busquet's (1921) experiments on isolated rabbit heart, and Reinberg's (1952) tests on isolated rabbit auricle. Takahashi (1933) in his studies of the isolated frog heart diluted Ringer's solution in various ways. He concluded that the lack of specific effect of sodium chloride and partly also that of sodium bicarbonate caused the decrease in rate. The changes in the amplitude resulted from the lack of specific effect of calcium chloride, potassium chloride and sodium bicarbonate. The reduction in osmotic pressure played no part in the decrease of the amplitude initially, but contributed

to it greatly at a later stage. Kruta (1938) concluded from his tests that calcium caused changes in the optimum rate of the guinea pig's left auricle. Addition of potassium, according to him (1949), caused slowing of the rate of the right auricle, regardless of the temperature.

Winkler *et al.* (1938) injected isotonic potassium chloride intravenously into a dog. Increased potassium concentrations in the blood resulted in the following changes, in order: *T*-wave alteration, depression of the *S-T* segment, intraventricular block, disappearance of the *P* wave, and cardiac arrest. Lenzi and Caniggia (1953) injected many different cations intravenously into a tortoise, seeking to determine their specific effects on the electrocardiogram. With the aid of ions Garb (1951) produced changes in the electrogram of isolated papillary muscle. Unghváry and Obál (1942) allowed several electrolytes (e.g. sodium chloride, hydrochloric acid and sodium hydroxide) and organic substances to act directly on the frog's heart in various concentrations. The criterion of the effect was the appearance of monophasic deformation, and they explained the mode of action by osmotic pressure and a specific ionic and molecular effect.

Many observers have reported on the favourable effect of *glucose* and also of saccharose on cardiac muscle. According to Noyons & Cousy (1923) and Belehrádek (1923), the frog's heart tolerates the substitution of glucose for the total amount of sodium chloride provided sodium bicarbonate is present. Belehrádek stated that saccharose, maltose, and above all galactose, were not equal to glucose in this respect. McDowall and Zayat (1952) expressed the view that the isolated right ventricle of a rat suffering from anoxia recovers if the sodium chloride content of Krebs' solution is reduced to one half and the osmotic pressure maintained with saccharose. In the tests of Masuoka *et al.* (1952) the addition of glucose caused a recovery in the amplitude of the contractions of an electrically driven strip of rat's ventricle after an initial decrease in amplitude. Fuhrman *et al.* (1950) and Kodama (1952) noted that in certain test conditions glucose promoted the oxygen consumption of heart muscle.

According to Mita (1926), non-electrolytes (saccharose, glucose and urea) exert upon the frog heart a specifical pharmacological effect, the reverse of the effect of the sodium and potassium ion. When

added as hypertonic solutions they stopped the heart beat in systole whereas sodium chloride and the other salts of Ringer's solution do this in diastole.

The results of the present study of the isolated right auricle of the rabbit are similar in many ways to the results reported by others. The effects of changes in the composition of the surrounding medium must be explained partly on the basis of variations in the osmotic pressure and partly on the basis of the specific effects of ions or molecules.

The *osmotic pressure* could be raised by two thirds from that in standard Locke's solution without appreciable changes in the *mechanogram*, but if doubled the effect was clearly injurious. A slighter change in the hypotonic direction caused distinct impairment. Reduction of the sodium chloride concentration down to 0.7 per cent by dilution with water strongly reduced the amplitude. But if the physiological level of osmotic pressure was maintained by adding isotonic glucose there was no reduction in amplitude even when the sodium chloride concentration was brought down to 0.5 per cent.

The rate was usually less impaired by changes in the composition of the fluid. Only in the test series in which the sodium chloride concentration decreased and glucose was added as a substitute for osmotic pressure was rate retardation observed without any essential change in amplitude; this was evidently due to the weakening of the specific effect of the sodium ion.

Addition of moderate amounts of glucose was also found to accelerate the rate and increase the amplitude. This effect appeared only in a relatively fresh preparation. This effect cannot be specific to glucose since the same result was achieved by adding hypertonic sodium chloride solution.

From the point of view of the *electrogram*, the *short-circuiting* effect of the electrolyte solution bathing the preparation was of prime importance. Changes in its composition affected both the amplitude of the recorded action potential and the conduction time. In addition, the ions and molecules exerted a specific effect on membrane. In air the amplitude of the *R* wave was much greater and the duration of the *QRS* interval longer than in fluid, because of the low electrical conductivity of air.

According to Hodgkin (1939), the conduction rate of isolated crab

fibre was 14—40 per cent faster in sea water than in oil. Håkansson (1954) stated that the propagation velocity of isolated frog muscle fibre was 20—50 per cent less in air than its velocity in Ringer's solution. Pond (1921) studied the conduction rate of skeletal and heart muscle in surrounding media of varying salt content and conductivity. He also concluded that the rate of conduction is in direct correlation to the electrical conductivity of the surrounding medium. Because of Pond's test arrangement Hodgkin did not consider his results completely conclusive, stating that changes in salt content may also cause alterations in membrane excitability. In studying the conduction rate of a nerve fibre, Hodgkin therefore kept the composition of the surrounding medium constant and the position of the electrodes unchanged, but the volume of the fluid was varied: thus the portions of fibre passing through the medium — sea water — varied. The greater the portion of fibre immersed in sea water the greater was the conduction rate.

In the present tests an increase in sodium chloride concentration alone led to reduction of the *QRS interval.* Andrus and Carter (1922) obtained the same result with turtle heart by changing the sodium chloride concentration of the perfusing fluid, and this reduction occurred in spite of a slowing of the rate. Lenzi and Caniggio (1953) also made the same observation after injecting sodium chloride solution intravenously into a tortoise.

The change caused in the *QRS* interval of the rabbit auricle by adding water was the reverse of that obtained by increasing the sodium chloride concentration. Prolongation of *QRS* was observed even before the rate slowed down. This can be explained in part by the impaired conductivity of the solution. It seems, however, that the injurious effect of the hypotonic solution on membrane function was the more important reason.

The short-circuiting effect of the fluid in contact with the heart on the *amplitude of the recorded action potential* is seen not only in isolated preparations but also *in situ.* Blood and tissue fluids have a similar effect. The tissues surrounding the heart are not quite homogeneous. Local tissue changes, due for example to a pathological process, may cause changes in the electrocardiogram. An intensified shunting effect is noted in pericarditis when a fairly large amount of fluid accumulates in the pericardium. When there is dilatation but no hypertrophy, the action potential apparently

decreases at once following powerful stress; in Schaefer's monograph this has been explained on the basis of increased blood content of the heart. Katz and Korey (1934) isolated the heart from the surrounding tissues on various sides. They concluded that the large vessels and their blood content did not play an important part. They thought the most important factor to be the contact between the heart and the posterior wall of the thorax. In foetal electrocardiography the short-circuiting effect of the amniotic fluid on the amplitude of the recorded action potential is a factor of practical importance, as appears for example from the study of Vara and Niemineva (1951). Yet, in the opinion of Southern (1954), this has less importance than the fat and muscle of the abdominal wall.— Belehrádek's (1923) observation that a greater potential difference is recorded in glucose than in Ringer's solution, because of the slower conductivity of the former, is in agreement with the present test series.

Throughout the experiments of this study the electrodes were in direct contact with the preparation, and the volume of fluid in the isolated organ bath was generally the same. Wilson *et al.* (1934) made tests on the turtle, placing one electrode distant from the heart and the other in contact with the heart, either directly on the ventricular surface or through a conductive medium. Whether the exploring electrode was near or in contact with the heart the form of the curve did not change although the height of the deflections varied. Buchthal *et al.* (1954), in their study of isolated frog muscle fibres, observed that the duration of the action potential was practically independent of the distance between the electrode and the muscle fibre. The amplitude, however, decreased to one tenth of the value recorded at the surface of the fibre when the electrode was moved to a distance of less than 0.5 mm from the fibre. Von Bonsdorff (1950) has also stressed that the distance between the electrode and the object is important from the point of view of the size of the action potential.

Merunowicz (1875) was evidently the first to observe the favourable effect of an alkaline *reaction* on cardiac activity. According to Martin (1905), a heart strip from a cold-blooded animal contracted more vigorously and longer in alkaline solution, even in conditions of anoxia, and he thought that alkali promoted the utilization of oxygen stores. The fact that alkalinity accelerates

the heart rate of cold-blooded animals has been reported by Mines (1913), Clark (1920), Andrus & Carter (1924), and Sands & Amberson (1928). The force of contraction is stronger in alkaline solution. Acidity reduces it greatly, as was shown by de Burgh Daly & Clark (1920) and Bogue & Mendez (1930). The hydrogen ion concentration has both a lower and an upper limit between which cardiac activity is possible. Komiyama (1929) stated that the heart of a frog ceased to contract at a pH of 6.0 and 12.0, but revived in both cases. Irreversible changes occurred at pH 5.0. In his opinion the hydrogen ion concentration affects the cell membrane as long as the changes are reversible. According to Del Castillo-Nicolau *et al.* (1951), the amplitude of the mechanogram of the frog heart began to rise at pH 3.0; it rose at first abruptly, then more slowly, until the pH range 9.0—10.0 was reached, and after this there was an abrupt fall. Increase of the potassium concentration reduced the pH range within which activity is possible. The heart shows fairly low sensitivity to a change of pH value alone. These authors consider that the effect of changes in pH may take place via the ferment system.

Fredericq (1925) reported that the chronaxia of the tortoise heart was prolonged when an acid perfusion fluid was allowed to exert its effect for a given period. In his opinion this was probably not due to the superficial effect of hydrogen ions but to a slow penetration into the protoplasm, though the change was a reversible one. Di Palma and Mascatello (1951) studied the isolated heart muscle of the cat, finding that a change in pH towards acidity lowered the resting excitability and prolonged the refractory period. Del Castillo-Nicolau *et al.* (1951) showed that the rheobase of the frog's heart did not change essentially within the pH range 4.8—11.0.

In the present investigation the *pH range of optimal mechanical activity* of the isolated rabbit auricle *was 8.0—9.0.* At pH 7.0—8.0 and 9.0—10.0 activity was weakened. Outside these ranges there was no contractile activity, at least no prolonged one. The extreme pH limits given above caused no irreversible changes unless the preparation was kept very long at these values. Both the rate and the amplitude decreased as these limits were approached. The standard pH of Locke's solution was 8.2. Graham (1949) and Burn & Kottegoda (1953) used the same or nearly the same pH in studying

the rabbit auricle. Acierno and Di Palma (1951) had a lower pH and used a gas mixture containing 5 per cent carbon dioxide in addition to oxygen.

Many investigators have directed attention to the fact that the *conduction time* is prolonged as the pH changes towards acidity or that it shortens with a change towards increasing alkalinity. This was noted in cold-blooded animals by de Burgh Daly & Clark (1920), Andrus & Carter (1922), Sands & Amberson (1928), and Bogue & Mendez (1930). Andrus and Carter (1924) obtained the same result in isolated dog heart. Wiggers and Banus (1926, 1929) reported that a decrease in the blood pH of a dog to about 7.0 generally caused a prolongation of conduction time between the auricle and ventricular surface. Both hydrochloric acid and carbon dioxide produced a depression of conduction, but not lactic acid.

Mines (1913) reported a reversal in the *T* wave direction of the frog's heart by altering the pH value. Sands and Amberson (1928) made the same observation with turtle and stated that the *P* and *R* waves may also be reversed and that the curve of potential difference recorded in very acid solutions tended to assume the form of a negative monophasic wave. Unghváry and Obál (1942), in fact, used the appearance of monophasic deformation as a criterion in their electrolyte studies which dealt also with the effect of hydrochloric acid and sodium hydroxide on the heart muscle of the frog.

The *QRS interval* of the rabbit auricle scarcely changed when the pH of Locke's solution was raised from 8.2 to 10.0, although the mechanical activities showed great deterioration. *QRS*, on the other hand, was prolonged as the fall of pH approached neutral reaction. The observation agrees with those made on pH and conduction time in studying the cold-blooded and the mammalian heart. The amplitude of the *R wave* also remained unchanged when the reaction was altered in the pH range 7.0—10.0. Addition of hydrochloric acid and sodium hydroxide did not appreciably alter the short-circuiting effect of the solution.

4. *Anoxia.* — It seems clear that lack of oxygen must weaken the *contractile force* of heart muscle as it is a part of the organism requiring aerobic conditions. Abderhalden and Gellhorn (1920) called attention to this matter in their study on strips of frog heart muscle. The rate, however, is less sensitive. According to Bach-

mann (1927), the automatism of the frog's heart persists for days, even in complete anoxia. Gottdenker and Wachstein (1933) found that the life span of a strip of rabbit heart shortened under anaerobic conditions. Resnik (1925) stimulated a vagotomized dog's heart electrically under anesthesia and found that lack of oxygen increased the tendency to fibrillation. König (1927) and McDowall & Zayat (1952), among others, made observations regarding the stimulating effect of glucose on an anoxic heart. The effect of anoxia on the oxygen consumption of isolated heart slices has been studied by Fuhrman *et al.* (1950) and by Kodama (1952).

In the present study, anoxia first affected the contractile force of the auricle. For some time the force of contraction remained unchanged thanks to old oxygen stores, but it then decreased steeply. *Rate* retardation appeared considerably later than the decrease in amplitude. Sensitivity to anoxia depended greatly on the condition of the preparation at the beginning of the test and on temperature. If the preparation was in good condition there was at first some acceleration of the rate owing to the direct excitatory effect on the preparation. In man, Lutz and Schneider (1919) consider acceleration of the rate to be due to stimulation of the cardiac medullary centres by decreased oxygen tension.

Among *electrocardiographic* studies Resnik's (1925) experiments deserve mention: he used a group of vagotomized dogs and another group in which both stellate ganglia also had been removed. Anoxemia at first shortened but then prolonged the auriculo-ventricular conduction. Resnik considered this due to direct action on heart muscle. The *QRS* interval responded in the same way, although the changes were much smaller than those in the *P-R* interval. May (1939) induced oxygen deficiency experimentally in man and this resulted in lowering of the *T* wave and sometimes in depression of the *S-T* segment. Garb and Chenoweth (1953) showed that the electrogram recorded from the cat's isolated papillary muscle had a wider *R* wave and lower *T* wave in oxygen deficiency. Anoxia also caused inversion of the *T* wave in ventricular strips. Erk and Schaefer (1944) recorded a monophasic action potential directly from the heart of mammals with micro-electrodes. As a result of anoxaemia the duration of the total monophasic curve and its upstroke lengthened, the height diminished and the downstroke was less steep.

Anoxia also prolonged the *QRS* interval of isolated rabbit auricle. The change was not convincing during the first few minutes but later it was indisputable. At the same time the rate slowed down and the amplitude of the *R* wave decreased.

B. CONCLUSIONS

In comparing the electrograms recorded directly from an isolated auricle contracting in a volume conductor and electrocardiograms taken with limb leads, the difference in the conditions of test must be kept strictly in mind. Schellong (1926) even maintains that a curve recorded directly is not comparable to one recorded with limb leads, even though their shape may be similar. The former method records only the local process occurring at the site of the muscle element over which the electrode is placed, the latter the total event. A second fundamental difference, in Schellong's opinion, is the fact that the steepness of the deflections is a function of time in indirect recording. On the other hand, Weber (1939) and Rothschuh (1948) have stressed the similarity in form of the curves recorded directly and indirectly. Electrograms taken directly under varying conditions also differ from each other. Recording directly from an isolated auricle in Locke's solution is more closely related to indirect recording with limb leads than is a recording from a preparation in air. In addition, the possibility of differences between species should be remembered, especially when applying the results of animal experiments to man.

1. *The Effect of Mechanical Stress on the Spread of the Excitation Wave.* — In both the isotonic and isometric test series it appeared clearly that the *QRS interval* was prolonged by stretch. The reliability of this observation is increased by the fact that the results obtained in fluid were consistent with those obtained in air. As the preparation stretched, the lead points of the electrodes were more widely separated and the preparation became thinner. Prolongation of the *QRS* interval means that the time taken for the excitation wave to spread through the preparation is lengthened, but it still does not, as such, indicate a possible change in the conductivity of heart muscle tissue. According to Schaefer's monograph, the conduction system occupies about six sevenths of the *QRS* interval in man, and the

heart muscle elements occupy the remaining one seventh. If depolarization occurred quite simultaneously in all heart muscle elements, the *QRS* interval would be very short. If the conduction time in the heart muscle elements were doubled, the duration of the *QRS* interval would increase by only 15 per cent provided that there was no simultaneous retardation of conduction in the conducting system. Not even pathological changes can cause so great a prolongation of the conduction time in the heart muscle elements.

As the auricle stretched about one third of its length, the *QRS* interval was prolonged in about the same ratio. If the relative parts occupied by the conducting system and the heart muscle elements in the *QRS* interval were the same in the rabbit auricle as in the human heart, then it would seem that the prolongation of the *QRS* interval under the test conditions must be due in the first place to lengthening of the time required for the excitation wave to spread in the conducting system. However, this may not be assumed for the rabbit auricle. According to Gray's Anatomy very few, if any, Purkinje fibres are present in the wall of the right atrium.

If the conduction time in the heart muscle elements had changed as much as it has been shown to change in skeletal muscle as a result of stretch, the following hypothetical conclusions could be drawn. Assuming that the part of *QRS* occupied by the heart muscle elements is one seventh, the *QRS* interval would have been prolonged by about 5 per cent at the most owing to the effect of the heart muscle component. If the Purkinje fibres play no part, the prolongation of *QRS* would be about proportional to the stretch, as is indicated by the results of the stretching and loading tests. It is possible, of course, that the time required for spread of activation is prolonged both in the conducting system and in the heart muscle. But it is not possible to estimate the relative parts of both the above components in the origin of the *QRS* interval only on the basis of prolongation of *QRS*.

Stretching did not by any means affect all muscle elements of the auricular preparation equally strongly. Nor was the stretch always longitudinal because the structure of heart muscle is syncytial and the muscle layers are not parallel. True, the auricle was cut so that most of the thick musculi pectinati were longitudinally directed. The *QRS* interval and stretch were related in such a way

that the former was prolonged linearly with the stretching force and not with the length of the preparation. The relationship between the stretching force and the length of the preparation was as follows: The more the latter increased as a result of stretch the greater was the force required to produce a given increase in length. Hook's law does not apply here, nor — according to Christen (1911) — does it apply to skeletal muscle.

Although it was established that the time taken for spread of the excitation wave is prolonged, nothing can be said with absolute certainty regarding a possible change in propagation velocity. The explanation of the difficulties lies in the somewhat irregular form of the preparation, its syncytial structure, and the possible role of the conducting system in the spread of activation. The change, if it occurs, is certainly not great.

The change in the *area of the QRS complex* in the loading and stretching tests has been assessed with the object of throwing additional light on the part played by the conducting system and by the heart muscle elements in the prolongation of the *QRS* interval and at the same time of discovering the value of this method of study for interpreting the curves recorded under the test conditions used here. The results of the loading and stretching tests in fluid and in air differed essentially. In fluid, the area of the *QRS* complex remained unchanged. According to Schaefer this would mean that the elements are desynchronized and the spread of the excitation is prolonged in the conducting system, but not in the heart muscle elements. In air, the area of *QRS* increased, which indicates the special importance of the heart muscle elements in the prolongation.

The area of the *QRS* complex is affected by the direction of the elementary vectors which changed in some elements towards that of the stretching force, in others away from it. This arouses doubts as to whether a reliable picture can be obtained of the isolated auricle. Moreover, stretching of the preparation involves a change in the distance between the lead points. The change in the direction of the vectors and in the distance between the lead points is of course the same in fluid and in air, and so they do not explain the difference between the results obtained. To some extent the size of the recorded action potential may also be affected by a possible, but slight, gliding of the electrode and the surface of the preparation

in relation to each other, because the size and form of the electrode remain unchanged but the preparation as a whole stretches when loaded. This factor is also the same in fluid and in air.

It seems improbable that the time taken by the spread of the excitation wave in fluid would be prolonged only in the conducting system and not in heart muscle elements and, in air, also, or only, in muscle either in the latter only or in both, though the effect of the surrounding ions on the conductivity of the membranes should not be forgotten. Hodgkin (1947) showed that this conductivity is of great importance in an isolated nerve fibre. The short-circuiting effect of electrolyte solution is sure to be a decisive factor in causing *R*-wave diminution as the lead points are moved further apart owing to stretching. The difference between the results shows how much caution is required in drawing conclusions regarding electrical phenomena in an auricular preparation on the basis of changes in the area of the *QRS* complex. This, however, does not nullify the observation that stretch has a prolonging effect on the time taken for the excitatory process to spread in an auricular preparation.

In heart muscle the relationship of stretch and conduction time is of theoretical interest, chiefly from the point of view of the electrocardiographic changes in cases of *cardiac dilatation*. Clinical observations indicate that in dilatation of the heart the prolongation of the *QRS* interval is very slight and that the duration of *QRS* does not exceed the 0.10 sec limit considered pathological in man. Few experimental studies have been published on this subject, and the tests on skeletal muscle are not as such readily applicable to the *QRS* interval of the heart.

The preparation used in the present study — in which stretch was found to increase the time required for the spread of the excitation wave — is more closely comparable with the human heart than is a frog heart strip or skeletal muscle. It must of course be remembered that, as the stretching force acts in the direction of one axis only, the change in the form of the preparation differs from that when the heart — a hollow, muscular organ — contracts *in situ*. There is scarcely any difference, at least no qualitative one, between the function of atrial and ventricular muscle. On the whole atrial muscle, which is thinner, adapts itself more readily to various conditions than ventricular muscle.

The results of the tests support the opinion that prolongation of the *QRS* interval in cardiac dilatation must be considered possible. In the auricular preparation prolongation of the *QRS* interval was observed as a distinct reversible phenomenon even before the state of insufficiency, and it even continued after this stage. On the other hand, the tests showed that the prolongation of the *QRS* interval due to stretching is not very great. Conduction time during the state of insufficiency was about one third longer than when the preparation was neither loaded nor stretched. There is good reason also, when evaluating the time taken for the spread of the excitation wave in cases of cardiac dilatation, to remember the effect of the vegetative nervous system on the conduction time and rate. As is known, exercise has been found to shorten the duration of the *QRS* interval in healthy persons — an aid to diagnosis recommended by Schellong (1932).

The work output of the preparation and the length of the *QRS* interval were not directly related. This is indicative of the separability of the electrical and mechanical events of heart muscle. Even during the state of insufficiency, the spread of excitation wave took but little less time than during the stage of maximal work output.

2. *The Effect of Temperature on the Spread of the Excitation wave and on the Duration of the Electrical Systole.* — The time required for spread of the excitation wave had its optimal temperature. When the temperature fell below or rose above 35°C, the *QRS interval* was prolonged. The two alternatives, since the nature of the events differs, should be dealt with separately in greater detail.

The temperature range of heart muscle activity is wider than the range of its spontaneous activity. Heat arrest of the heart was earlier attributed only to disappearance of the contracting capacity of the heart muscle. This opinion was presented by Stewart and by Ide, both in 1892. Later research has shown that the contracting capacity and electrical excitability still persist after arrest, especially if the arrest has been sudden. Unger (1913), Mangold & Kitamura (1923), and Meltzer & Mononobe (1928) have stated in explanation that the conducting system is more sensitive than heart muscle as a whole to the injurious effect of heat. The conductivity of Locke's

solution improves as the temperature increases, and yet the *QRS* interval of the auricular preparation was prolonged at above 35°C. This fact indicates that the conducting system is probably more sensitive than the other functional components of the preparation to the harmful effect of heat. The rate increased more and more, and at the highest temperature studied — 40°C — the function of the sino-auricular node still showed no signs of damage. Another possibility is that the prolongation of *QRS* at a high temperature is due to relative anoxia when the preparation is beating at a rapid rate. The result of the increased temperature was that the *QRS* interval occupied an increasing proportion of the cycle.

When the temperature fell below 35°C the *QRS* interval was prolonged and the rate retarded. Spontaneous contractions often ceased on approaching 20°C. The *QRS* interval lengthened as the temperature fell, but the degree of prolongation was smaller than could be accounted for by the slowing of the rate. The *QRS* interval occupied a smaller proportion of the cycle below than at 35°C. This being so, the proportion of the cycle occupied by the *QRS* interval increased with a rise in temperature within the temperature range studied. This shows that the function of the sino-auricular node as pacemaker of the heart and the conductivity of the preparation respond separately to the changes in temperature.

The *Q-T interval* recorded at various temperatures was most intimately related to the rate; its relation to cycle length remained unchanged at the temperatures studied. The time of spread of the excitation wave and the duration of the electrical systole thus responded differently to a change in temperature. The *Q-T* interval is determined by the duration of the excitatory process in the muscle tissue (excluding the effect of *QRS* on *Q-T*), and it is not essentially influenced by changes in conduction velocity. Del Castillo and Machne (1953) stated that the membrane resistance of skeletal muscle increased on cooling, the value for Q_{10} being 1.35. Judging from the relationship of *Q-T* and *QRS*, heart muscle tissue again proved less sensitive than the conducting system to temperature effects. Assuming that the prolongation of the *QRS* interval at a high temperature is due to anoxia, a shortening of the *Q-T* interval would be expected according to Erk and Schaefer (1944). The possible anoxia, however, is relative, and it may be concluded that the *Q-T* interval is less sensitive to anoxia than is

the *QRS* interval. The rhytmic action of the sino-auricular node and the duration of the electrical systole can be considered to follow the same course within the temperature range studied.

The changes observed in the activities of the isolated rabbit auricle support the view of Straub (1926) that the effect of temperature on the heart is not limited to the sino-auricular node; the total heart is involved.

3. *Concerning the Time Relations of Mechanical and Electrical Events.* — Observations were made regarding the time relations of the mechanical and electrical events in the stretching tests, the preparation contracting isometrically. The duration of the contraction period of the mechanical systole and of the *Q-T* interval did not change under varied stretch. Both occupied an unchanged proportion of the cycle at various temperatures. However, the *QRS* interval and the relaxation period of the mechanical systole were prolonged as a result of stretch.

As with the total heart, the process of depolarization, which began slightly before mechanical contraction, terminated with the onset of contraction. The peak of the mechanogram coincided with the peak of the *T* wave or came slightly after it. The greater the amount of stretch, the earlier in proportion did the *T* wave end, or repolarization cease, in the area of the downswing of the mechanogram.

The relationship between the duration of the contraction period and the *Q-T* interval and their relation to cycle length remained unchanged in spite of changes in the force of contraction and temperature. The duration of relaxation seemed to be related to the force of contraction; both increased with increased stretch and a falling temperature. Because the duration of the electrical systole was not the same as that of the mechanical systole, the duration of the relaxation period is evidently comparatively independent of repolarization in regard to time.

The fact that retardation of depolarization caused no prolongation during the relatively much longer contraction period also indicates that the mechanical and electrical events are to a certain extent independent of each other. The independence of depolarization as regards the duration of the contraction period was further illustrated by the circumstance that the prolongation of the *QRS*

interval at a high temperature did not prolong the contraction period in relation to cycle length.

The duration of the contraction period is evidently determined by the mode of action of the muscle tissue on the »all or nothing» basis. The changes in the time required for the spread of the excitation wave within the limits in which such changes occurred in loading and stretching tests, are not able to influence the duration of the contraction period. The heart muscle obeys the command in the time required at the prevailing temperature, although the time taken by the command to reach its destination and the force generated in fulfilling the command vary.

After a more vigorous contraction the relaxation period lasted longer, though depolarization was completed as rapidly as after a weaker contraction. Repolarization, which is not affected by the conducting system but solely by the properties of the heart muscle tissue, follows its own schedule independently of the force of contraction and has its own characteristic speed at each temperature. Whether the prolongation of relaxation is due to changes in metabolism or other functions, and if so what their nature is, or whether this phenomenon is caused by the part of heart muscle which is passive in relation to the contractile activity of heart muscle, are questions which are outside the scope of this investigation.

4. *Effects of the Surrounding Medium on the Electrogram.* — Attention was paid to the *amplitude of the action potential* recorded in the test series in which the composition of the solution bathing the auricle was changed and no loads were used, as well as in the loading and stretching tests in which the composition of the fluid was kept unchanged throughout the test series.

A striking observation in the loading and stretching tests is that the change occurring in the amplitude of the R wave in fluid differed in direction from that in air. In fluid, as will be remembered, it decreased, and in air it increased up to a certain limit with increased stress. This is in keeping with Seeman's (1913) finding that in the curve recorded from an isolated frog heart the R-wave change caused by increased pressure of the perfusing fluid depended upon the electrolyte content of this fluid. The R-wave prolongation observed in air resembles the result obtained with skeletal muscle. The effect of the amount of fluid contained in the heart cavity is

also distinct, although the quality of the fluid remains unchanged. This shows how difficult it is to give a definite explanation of the changes in the size of the action potential both in tests and in clinical cases in which the mechanical tension of heart muscle increases together with the blood content of the heart and intracardiac pressure; the effect of the different factors may be conflicting.

The addition to Locke's solution of sodium chloride, glucose and water in various concentrations resulted in distinct changes in the *R* wave. The electrolyte content of the solution was of decisive importance for the amplitude of the *R* wave, even though the change in the surrounding medium could not have failed at the same time to affect membrane properties such as the electrical resistance. These last effects appeared most clearly after the changes in the composition of the fluid had reached a maximum, and symptoms directly due to injury of the preparation became more prominent. The enlargement of the *R*-wave amplitude was most pronounced when the preparation was transferred into insulating air.

Lenzi's and Caniggia's tests on the tortoise bear an interesting resemblance to those of the present author: they injected sodium and lithium ions intravenously, causing a marked decrease in the amplitude of the *R* wave. As this phenomenon was not produced by certain other considerably more toxic ions, they denied the possibility of a short-circuiting effect and regarded the phenomenon simply as a specific effect of the sodium and lithium ion. It must be asked, however, whether the specific effect is the only one. Potassium, rubidium and cesium ions can scarcely be injected in as large amounts as sodium and lithium ions.

Many factors are responsible for the sensitive variation of the amplitude of the action potential recorded. An extremely important part is played in this respect by the primary electrical phenomena in the membrane and the specific factors influencing these phenomena. The composition and character of the surrounding medium or perfusing fluid and the distance between the electrodes and the preparation are also important factors.

The conductivity of the surrounding medium has an effect on the size of the potential and also on the *rate of spread of the excitation wave*. The *QRS* interval was about 30 per cent longer in air than in standard Locke's solution. In evaluating the results when the com-

position of the solution was modified, it was not only the change in the conductivity of the solution itself that had to be borne in mind but also the effect of the altered conditions on the conductivity of the preparation, that is on the electrical membrane resistance, as Hodgkin has stated regarding nerve fibre. The shortening of *QRS* following an increase in sodium chloride concentration was an interesting observation. It is difficult, of course, to evaluate the part played by each factor involved in this phenomenon. A specific change, probably due to potassium, was observed when potassium chloride was added simultaneously with sodium chloride and calcium chloride in the same ratios as these are present in standard Locke's solution: there was a reversal of the *QRS*-shortening caused by the addition of sodium chloride alone, and the final result was that the *QRS* remained unchanged in spite of a great diminution in the *R*-wave.

5. *Effect of Anoxia on the Electrogram.* — The prolongation of the *QRS* interval in anoxia may conceivably be due to retardation of the spread of the excitation wave in either the conducting system or the heart muscle tissue, or both. If this question can be illuminated at all on the basis of changes in the area of the *QRS* complex as recorded from a heart strip in Locke's solution, anoxia tests should help to solve the problem; the load remained unchanged throughout each test series and there was no essential variation in the distance between the lead points. The area of the *QRS* complex remained unchanged. According to Schaefer's interpretation, the retardation of the spread of the excitation wave must have taken place predominantly in the conducting system.

Against this, the conducting system of mammals has been found to consume less oxygen than heart muscle tissue, as was shown by Buadze & Wertheimer (1928) and Kolmer & Fleischmann (1928). On the other hand, it is known that anoxia of the total heart causes, in particular, a retardation of conduction in heart muscle tissue and probably at the same time in the conducting system, as Schaefer pointed out in his monograph. The situation must be different in the case of an isolated auricle or former conceptions must be erroneous, or, the area of the *QRS* complex is not a reliable criterion.

Since anoxia in the tests was carried sufficiently far for spon-

taneous activity to cease completely, it is clear that the effects of oxygen deficiency cannot be limited to one factor. The gradual decrease in the amplitude of contraction and the final cessation of sinus node activity show that each form of activity was affected. It seems difficult in the face of this to maintain that conduction velocity is not retarded in heart muscle tissue. In addition, anoxia is a factor which impairs the activity severely; it may influence the properties of the membranes and thereby contribute to a decrease in action potential, which in turn may compensate the effect of the factors increasing the area of the *QRS* complex. A decrease of action potential resulting from anoxia has been noted by Trautwein *et al.* (1954) in the papillary muscle and Purkinje fibre of mammals.

SUMMARY

The mechanical and electrical phenomena in the isolated right auricle of the rabbit contracting spontaneously in Locke's solution and in air have been studied. The preparation was subjected to mechanical stresses at various temperatures: loading which resulted in isotonic contractions, and stretching which resulted in isometric contractions. The electrolyte content of the surrounding medium, the osmotic pressure and the hydrogen ion concentration were varied by modifying the concentrations of the constituents of Locke's solution. Anoxia was induced by substituting nitrogen for oxygen.

A change in the amount of *mechanical stress* did not influence the rate. The work performed by a single isotonic contraction and the force generated by an isometric contraction increased to a certain definite limit with increasing stress, to fall again afterwards, the former slowly, the latter more rapidly. As the rate was unaffected by the amount of stress, the curve showing the work output in the isotonic tests and that showing the product of the rate and the contractile force in the isometric tests are similar to the curves indicating the work performed by, and the force of, a single contraction. The duration of the contraction period of an isometric contraction remained unchanged, but relaxation was prolonged with increased stretch.

The *QRS* interval increased proportionately with the stretching force. It seems possible that the prolongation of the time taken for spread of the excitation wave was due chiefly to its prolongation in the conducting system. The duration of the *Q-T* interval was not affected by an increase in stress.

A rise in *temperature* caused acceleration of the rate: Q_{10} varied from 2.6 to 2.2 in the temperature range 25°—40°C. The

optimal work output was obtained at the temperatures of 30° and 35°C, i.e. below body temperature.

The *QRS* interval was shortest at 35°C. However, the lower the temperature, the shorter was the *QRS* interval in relation to cycle length. The fact that *QRS* was again prolonged above 35°C shows that the conducting system was more sensitive than muscle tissue to the injurious effects of heat or that the depolarisazion process was prolonged by a relative anoxia. The proportions of the cycle occupied by the contraction period of the mechanical systole and by the *Q-T* interval remained unchanged within the whole temperature range studied, although the duration of the relaxation period grew proportionately longer as the contractile force increased with decreased temperature.

The *composition of the surrounding medium* could be changed within wide limits without impairing the activity of the isolated auricle. Addition of sodium chloride and of glucose caused no appreciable changes in mechanical activity when the osmotic pressure was raised by two thirds of that of standard Locke's solution. Not until the osmotic pressure was doubled was a marked reduction in amplitude observed. The rate was less sensitive to these changes. Activity ceased rapidly when the sodium chloride concentration fell below 0.8 per cent, provided the osmotic pressure was not maintained with glucose. The tests in which the solution was diluted with isotonic glucose showed that the arrest was not due to a specific lack of sodium or chlorine ions. The optimal pH range was 8.0—9.0. Activity continued within the pH range 7.0—10.0.

The effect of the surrounding medium on the electrograms was due partly to changes in its own electrical conductivity, the result of the electrolyte content. On the other hand, the specific and unspecific direct and indirect effects of the different constituents on membrane activities must be taken into consideration. The *QRS* interval was about 25 per cent shorter in Locke's solution than in air. Addition of sodium chloride to Locke's solution shortened the duration of *QRS*. This shortening was prevented by adding potassium and calcium chloride at the same time, probably specifically by the former. When the solution was made hypotonic by adding water, the *QRS* interval was greatly prolonged. The sodium chloride concentration, however, could be reduced to 0.5 per cent

before prolongation ensured provided the isotonicity of the solution was maintained with glucose. Prolongation of *QRS* was observed when the pH approached the neutral reaction.

The electrical conductivity of the surrounding medium affects the size of the recorded action potential greatly. The amplitude of the *R* wave in air was many times that in Locke's solution. The *R* wave diminished on adding electrolytes and rose when the electrolyte content was decreased. Changes in membrane activities must also be considered here. The height of the *R* wave was not essentially affected by changes in hydrogen ion concentration.

Anoxia soon resulted in failure. The amplitude of a single contraction was more sensitive than the rate to the effect of anoxia. In anoxia, the *QRS* interval was prolonged, the height of the *R* wave reduced, and the rate simultaneously retarded.

A comparison of the phenomena related to the activity of the isolated auricle with those of the total heart, whether isolated or not, or those of skeletal muscle, shows both similarities and differences. They lie chiefly in the fact that the activity of the isolated auricle is controlled by a simpler regulatory mechanism. The right auricle represents a unit capable of independent function; its sub-units, such as the pacemaker and the contractile muscle tissue, show their own peculiar properties as the experimental conditions change owing to various factors. Many details of the results of this study are easily explained by assuming that the conducting system also plays an independent part in the function of the isolated rabbit auricle. Although the changes in the electrogram were very often divergent from those in the mechanogram, the electrical and mechanical events cannot be regarded as entirely independent of one another. The effect upon the electrogram of the conditions of recording is of the utmost importance, not only from the point of view of magnitude but also of form.

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